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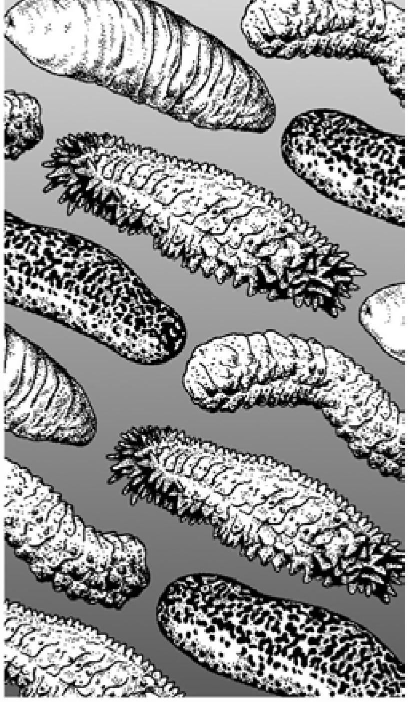

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Editorial

The 37th issue of the *SPC Beche-de-mer Information Bulletin* has 23 original articles and reports on the biodiversity, fisheries, aquaculture and trade of sea cucumbers in various regions, including Papua New Guinea, Philippines, China, Brazil and Nicaragua; sea cucumber symbionts; the reproduction of some sea cucumber species; and the development of methods to better study and rear them. New *in situ* observations are also included in this issue, along with communications about upcoming conferences, and PhD dissertations that were completed this year.

The first article is from K. Barclay et al. who summarise some of the major findings of a governance analysis that was conducted to assist the National Fisheries Authority of Papua New Guinea with understanding the factors influencing the effectiveness of their revised Beche-de-mer Fishery Management Plan.

The second and third articles are from the Philippines. R. Dolorosa et al. determine the species diversity of sea cucumbers in Rasa Island Wildlife Sanctuary in addition to the catch-per-unit-effort and earnings of collectors. J.B.S. Jontila et al. provide an initial assessment on the status of sea cucumbers at Arreceffi Island in terms of species composition, abundance and distribution.

Fisheries information comes from Turkey, Brazil and Nicaragua. M. Aydın reports on the present status of the sea cucumber fishery in Turkey and J. de Souza Junior et al. on sea cucumber fisheries in northeastern Brazil. Rogers et al. draw our attention to a fishery in Nicaragua that operates from nearby Honduras, where unethical conditions occur aboard fishing vessels and where desperate poverty drives fishers to work in dangerous conditions.

S.W. Purcell and M. Wu reveal that the sandfish *Holothuria scabra* is now produced in southern China within multitrophic polyculture ponds with pearl oysters and groupers. S.W. Purcell et al. record the values of the sea cucumber "pentard" in China and determine whether market price varied as a function of the size of the products.

Works on sea cucumber reproductions come from R. Santos et al. and from V. Agudelo-Martínez and A. Rodríguez-Forero. The first team characterizes the reproductive biology of *Holothuria mammata* from Peniche, Portugal, and the second that of *Isostichopus* sp. aff *badionotus* from the Carribbean coast of Colombia.

Two original articles focus on sea cucumber symbionts. J.F. Hamel et al. reveal a new host for a species of the genus *Anoplodium*, and report on its infestation rates and negative impacts on the host, supporting its classification as parasitic. K.W. Mueller present the symbionts of the giant red sea cucumber, *Parastichopus californicus*, in Washington State (USA), with some implications for culturing the host.

F. Ducarme provides an update on the geographical range of the holothurian species *Actinopyga capillata*, first described as endemic from the Mascarene Islands, with one ectopic record from the Philippines. D. Pratas et al. show the results of four tests that aimed at determining the gender of *Holothuria forskali* without causing evisceration or consequential mortality.

This issue also reports on the processing techniques of teatfish in Fiji, on field observations on the regeneration of *Synapta maculata*, and on sea cucumbers from La Réunion, Belize and Sulawesi. The last section of this issue includes communications about workshops and conferences that were held in 2016 and some that will take place in 2017. Congratulations are expressed to Georgina Robinson from Newcastle University (United Kingdom), Guillaume Caulier from the University of Mons (Belgium), Marielle Dumestre from the University of Hong Kong (China) and Kennedy Wolfe from the University of Sydney (Australia) who recently completed their PhD dissertations of various aspects of sea cucumbers.

Igor Eeckhaut

P.S: In line with a worldwide trend to limit the impact of producing printed publications on the environment, SPC has decided to stop the production and distribution of printed copies of this and other information bulletins. The BDM bulletin is now only produced in digital format and remain accessible from SPC's website at:

http://www.spc.int/coastfish/en/publications/bulletins/beche-de-mer.html

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Governance and the Papua New Guinea beche-de-mer value chain

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Abstract

The sea cucumber fishery was a highly significant fishery for many years in Papua New Guinea, supplying 10% of the global trade in the mid-2000s. However, increasing prices and an influx of buyers entering the trade has led to overfishing of sea cucumber stocks, and a moratorium was subsequently introduced by the National Fisheries Authority in September 2009. This paper draws out key findings from an analysis of the governance arrangements along the beche-de-mer supply chain from village-based fishers and suppliers in Papua New Guinea through trading to end markets in the Peoples Republic of China. We give an overview of the value chain, showing that fishers were doing quite well from the sea cucumber fishery compared with exporters in the sellers' market operating in the years before the moratorium, and the deeply opaque nature of import markets via the Hong Kong "grey trade". The analysis of management measures shows that while ecolabels seem an unlikely measure in the short term, there may be opportunities for branding according to place of origin, quality and food safety. We find that the regulation of exports rather than the fishery *per se* is an excellent fit between the operational realities of the business and government capacity. This did not, however, prevent overfishing before 2009, but it nevertheless provides a model with great potential for other countries with similarly structured fisheries and trade to consider.

Introduction

The objective of this study was to conduct a governance analysis to assist the National Fisheries Authority (NFA) of Papua New Guinea (PNG) and other stakeholders to grasp the factors influencing the effectiveness of the revised National Beche-de-mer Fishery Management Plan (herewith, the Management Plan), which was gazetted in September 2016 in preparation for the reopening of the PNG sea cucumber fishery in 2017. We employed an "interactive governance" approach, wherein ideas from governance studies have been developed for use in fisheries management (Kooiman et al. 2005; Jentoft and Chuenpagdee 2015). In this approach, all of the factors affecting the governance of a fishery – the ecology of the fishery, government, non-governmental organisations, social values and markets – are considered. To encompass markets, the approach uses the concept of a "fish chain", which is similar to a supply chain or value chain except that those concepts are largely economic, whereas the interactive governance goals are multidisciplinary (i.e. they consider other factors such as resource sustainability, food security, community well-being, livelihood viability and social justice). Furthermore, interactive governance influences are considered at the various scales relevant to the fishery, from local to provincial to national and global. Because this kind of broad governance analysis has not been done for sea cucumber fisheries before it was appropriate to take an exploratory approach, for which qualitative methods are best suited (Barclay et al. 2016a). On the basis of this broad research it is possible to narrow down questions for future research using quantitative methods. Data consisted mainly of interviews with fishers and traders in PNG and the Peoples Republic of China (hereinafter, China), sea cucumber scientists and policy-makers in various countries, and an extensive literature review. In this article we summarise some of the major findings of the project that may be of interest to sea cucumber fisheries managers and researchers. We focus on elements of the fish chain from PNG to Asian markets that are less well-understood and then highlight some features of governance. Readers interested in further details can download the full 168-page report (Barclay et al. 2016b), posters and a brochure from the University of Technology, Sydney website.[3]

The "fish chain"

The sea cucumber fishery in PNG prior to the moratorium in 2009 was extensive; it was carried out in most coastal and island locations around the country, and involved more than 26 species of sea cucumber, from very high-value to very low-value (Kinch et al. 2008). It was a small-scale informal fishery with multiple landing points, conducted

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[3] http://www.uts.edu.au/about/faculty-arts-and-social-sciences/research/projects/png-beche-de-mer-fish-chain 2016

Figure 1. Papua New Guinea.
Note: WNB = West New Britain; ENB = East New Britain; Nth = North).

mostly from shore, canoe or dinghy by indigenous Papua New Guineans (i.e. no foreign fishing fleets, although illegal Vietnamese fishing vessels have become a recent problem, with several vessels arrested in the East Sepik, New Ireland and Milne Bay provinces). Women, men and children fished close to shore and gleaned in shallow areas. Young men dominated the fishing, which they conducted farther from shore or which involved deep diving. Some level of processing was done by fishers – minimal processing, usually first boiling only if the beche-de-mer could be sold quickly, full drying if fishers had to wait some weeks to be able to sell their product because they were in remote locations and thus needed to wait for suitable transport. Exporters were based in provincial capitals or Port Moresby and bought the beche-de-mer through buyers who travelled out to fishing areas or fishers who brought product to them to sell direct. There were a handful of exporters in each maritime provincial capital and more in Port Moresby, which meant the export node of the supply chain was much more consolidated than the fishing node.

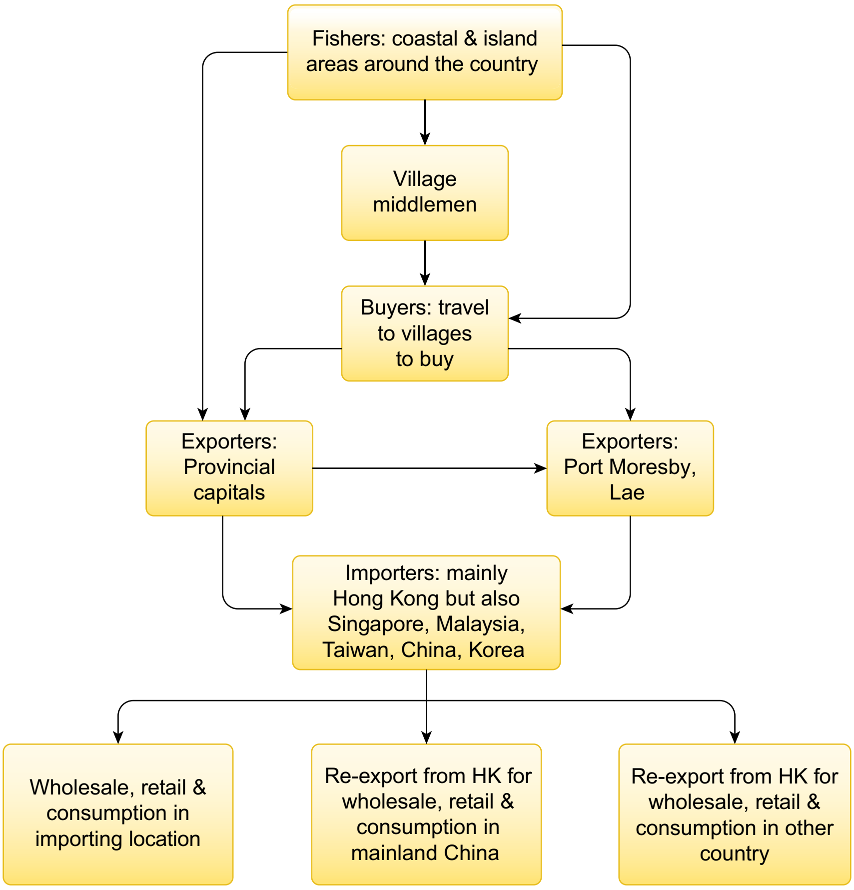

Figure 2. Papua New Guinea beche-de-mer fish chain.
Note: HK = Hong Kong.

Regional markets for beche-de-mer have existed for centuries, and they have expanded greatly since the 1980s due to growing affluence in China. Beche-de-mer was exported mainly via Hong Kong Special Administrative Region (hereinafter, Hong Kong) as an *entrepôt* to other regional markets (Kinch 2004, Kinch et al. 2008). Most of the PNG product was bound for new markets in mainland China, with some high-value product retained for established high-end markets in Hong Kong or Singapore. The tropical species of sea cucumbers exported by PNG are consumed mostly in southern China, with much smaller amounts sold to markets in northern China. While there is a diversity of trade routes within mainland China, one key route is from Hong Kong to Guangzhou, and then to other centres within mainland China. Hong Kong, as a luxury seafood hub for the Chinese market, is a free port with no tariffs, while tariffs as high as 30% (depending on China's trade relations with the export country) for import into China. So, although the trade from Hong Kong into China is extensive, it is a form of "grey trade" (that is, illegal). Our interviewees said trade via Vietnam, highlighted as a route by other researchers (Conand et al. 2014), was not significant, but it should be noted that due to the illegal nature of the trade, our interviewees were reluctant to discuss details of how beche-de-mer moved from Hong Kong to mainland China.

The beche-de-mer trade, from buying from villagers through export to import and retail, was highly dependent on relationships. Rather than arms-length, contract-bound market arrangements, trading was almost entirely based on relationships and trust. The exporters we interviewed were unanimous in saying that their relationships with importers were key to being able to operate as a beche-de-mer exporter, and to how well they did in the business. Relationships with importers affected the prices offered. One exporter related how, despite family connections to China, it took him years to learn what the market rates were and thus be able to charge the best price. Different exporters would get different prices from the same importers for the same quality product, depending on the strength, longevity and honesty of the relationship. All of the importers in Hong Kong and Guangzhou that we interviewed also stressed the importance of working with trusted local business partners from PNG.

Before the PNG sea cucumber fishery was closed in 2009 it was effectively a "seller's market". Most Papua New Guineans interviewed felt that exporters were making the best deal from the fishery, because in PNG and other Pacific Island countries it is normally assumed that "foreign" business people (most exporters not of indigenous PNG ethnicity) exploit village producers, and in many cases this assumption, with roots in the colonial experience, has a basis in fact (Barclay 2012). Figures from 2007, however, show that sea cucumber fishers were getting more of the final market price than exporters (Kinch et al. 2007, 2008, see Fig. 3). We speculate that fishers were able to get such good prices because from around 2004 there were so many exporters competing for supply in PNG that it pushed prices up. It was also possibly due to the fact that PNG fishers avoided being captured in patron–client relations with traders. Such relations have been found in other small-scale fisheries in developing countries to depress prices (e.g. Padilla et al. 2003). The reasons PNG fishers were able to gain such good prices is important to follow up in future research because many sea cucumber fishers globally are economically marginalised. Fishers captured more of the final retail value of their beche-de-mer when it was of higher quality

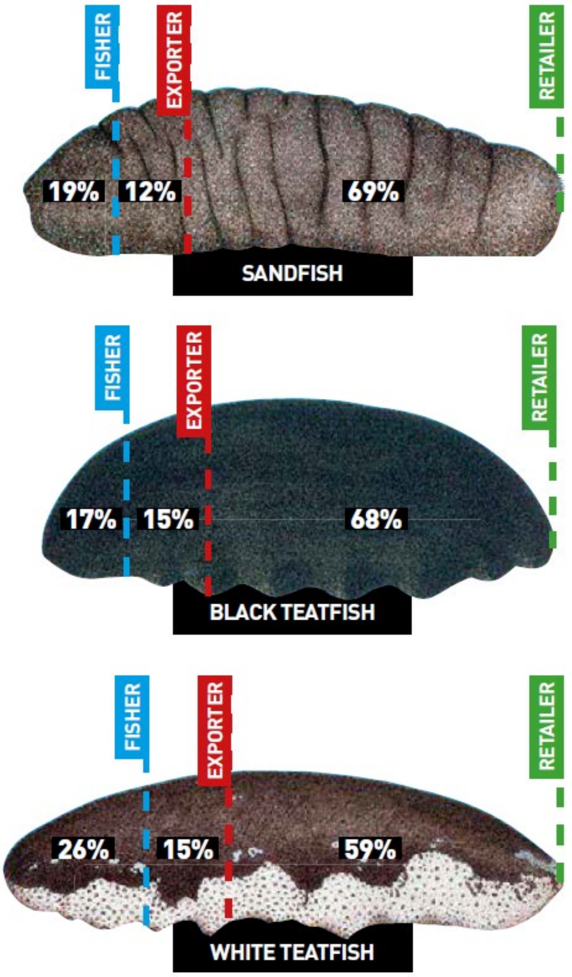

Figure 3. Market prices for fishers, exporters and retailers in 2007 (Kinch et al. 2007, 2008). Sandfish (*Holothuria scabra*), black teatfish (*H. whitmaei*), white teatfish (*H. fuscogilva*).

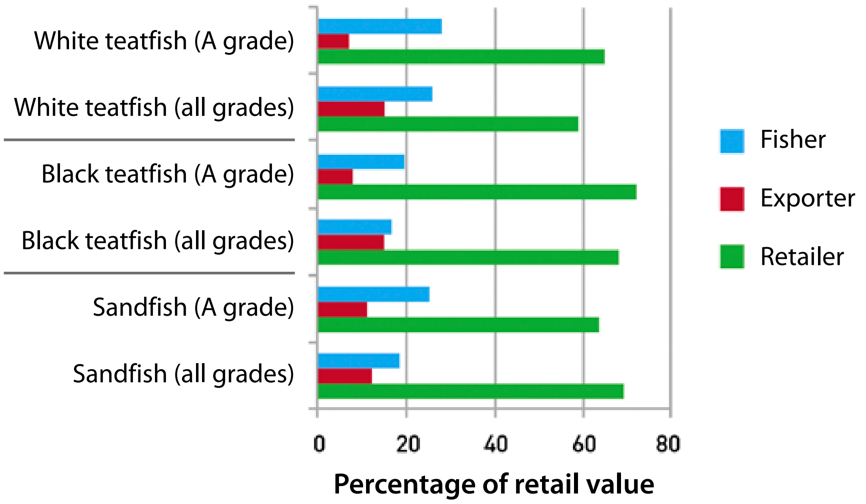

Figure 4. Market price variations by grade in 2007 (Kinch et al. 2007, 2008). White teatfish (*H. fuscogilva*), black teatfish (*H. whitmaei*), sandfish (*Holothuria scabra*).

(Kinch et al. 2007, 2008, see Fig. 4), as has been found in other studies (Purcell 2014). The 2007 figures also show that there was a substantial increase in value after the beche-de-mer was imported to Hong Kong. A more recent study on prices from other Pacific Island countries (Purcell 2014) had similar findings – the retail price in Hong Kong is, on average, 2.7 times the export price across all the species traded.

Many fishers expressed a desire to sell directly to importers, and several exporters also advised that they had tried to "shorten the chain" and capture more of the value between them and the retail node of the chain (see Fig. 3) by selling directly to retailers. These exporters had been unable to do so, however, because the retailers they contacted were unwilling. Moreover, the importers we interviewed expressed no interest in cutting out exporter companies and going direct to fishers. The Hong Kong-based traders interviewed felt it was necessary to go through PNG exporters because of the need for what they cited as relationships with the fishers. In addition, only PNG citizens could hold an export license. According to traders, the fishers needed close liaison to produce quality product, and the PNG exporters based in provincial towns were able to liaise with fishers in a way overseas-based importers could not. Other traders spoke specifically of the difficulties of working in PNG: one advised that the security situation was very poor so he was not keen to invest there, while several more simply stated that they had heard stories of traders being cheated there and that they did not trust the local people. Even buying through local exporters, however, required building up trust first; without trust, such an undertaking was almost unanimously viewed by traders as too risky and dangerous. This point was emphasized by almost every trader interviewed.

Governance

There are many different opportunities for governance along the entire fish chain discussed in the full report for the project (Barclay et al. 2016b). We briefly discuss here two noteworthy sets of challenges and opportunities for improved governance that arise within the consumer end market of China and PNG's revised Management Plan.

Internationally, there are some potential measures that could influence governance of the fishery, including the possibility of listing overfished species under the Convention on the International Trade in Endangered Species (CITES), although sea cucumbers, despite a submission by the United States for listing on Appendix II, did not get much mention at the 2016 CITES Conference of Parties meeting in South Africa, as sharks were the primary focus. Greater scrutiny of imports by the Chinese government is another potential influence. However, the illegal nature of the beche-de-mer trade from Hong Kong into its major markets in China poses a clear obstacle to effective governance. Bringing the trade between Hong Kong and the rest of China into the legal sphere is a prerequisite for improved data on the trade all the way along the supply chain, the kinds of governance benefits that could arise from having traceability, and potential trade related measures such as anti-illegal, unreported and unregulated documentation being required by China as an importing market state.

Market-based measures

In general, market incentives for sustainability are not yet significant in the main Chinese markets. Traders interviewed in Hong Kong and China acknowledged the problems of resource sustainability in sea cucumber fisheries, and that this was leading to beche-de-mer being more difficult to source from some locations. Many of the traders in Hong Kong and Guangzhou knew there was a moratorium on the PNG fishery and why it was in place. One Hong Kong trader who had had long dealings with PNG exporters was very supportive in principle of the need to manage PNG's sea cucumbers, saying that this was "good for the country" and "good for the livelihoods of villagers". Other traders agreed with the broad notion that fisheries should be sustainably managed, suggesting that it could help to stabilize prices. However, most traders were unwilling to seriously engage with sustainability actions and initiatives, and did not feel that doing so would improve their business, especially as the Chinese seafood industry is sceptical that anyone will pay more for ecolabeled product. Interviews with exporters in PNG supported these findings, with all exporters interviewed saying none of their importers had ever raised resource sustainability or ecolabeling as something they were concerned about. Others viewed sustainability as solely the responsibility of exporting producer countries and did not see the role of traders relating to governance to ensure resource sustainability. This is not to say that ecolabeling will never take off in China, but it indicates it is not a short-term proposition (see Fabinyi 2016 for a summary of issues relating to sustainable seafood consumption in China).

In contrast, consumer preferences for food that is considered safe and healthy significantly overshadow concerns about sustainability that may be present (see Fig. 5). At the high price marketing end, food safety and quality concerns could potentially be linked to ecological factors and sustainability issues. There is potential for PNG product to be marketed as coming from "pristine" South Pacific waters without the industrial or sewerage pollution found in many Asian source countries. Currently, however, PNG's reputation in end markets for poor or unreliable quality beche-de-mer is a problem. Managers in PNG's NFA who were interviewed for the study said they are aware of the negative reputation with PNG product and are considering methods of extension for improving quality to increase the value of the fishery alongside efforts to improve sustainability (with higher prices, fishers could still have a good income even if catches are reduced).

Regulating exports

In PNG, our analysis found that, on the whole, the revised Management Plan is a good option: it is pragmatic and based on a thorough understanding of how the fishery operates.[4] The new emphasis on devolution, for example, is a good compromise between the need to allow for the delegation of responsibility where particular provincial governments and local organisations demonstrate preparedness for it, but not to rely on devolution where capable institutions are not yet in place. In particular, when we look at the complexity and scale of the beche-de-mer fish chain, the PNG government's choice to regulate exports is a good option. The fishery is extensive, informal and conducted in areas where government services are minimal. One international study has found that more sustainable sea cucumber fisheries correlate with certain kinds of fisheries management (Purcell et al. 2013). With an extensive and informal fishery in PNG, however, vessel controls and limited entry to the fishery are not feasible, and enforcement capacity at the fishery level is a long way from being achieved. Cash-earning opportunities are extremely limited in villages far from transport routes because of the expense of bringing inputs in and sending goods out, so the pressure to fish sea cucumbers will remain very high. At the point of exporting, on the other hand, beche-de-mer supply is consolidated from hundreds of thousands of fishers down to a handful of exporters in each maritime provincial capital and in Port Moresby. Exporting is a formal business requiring government licensing. Moreover, it is conducted in towns where government services are functioning, and

Figure 5. Sea cucumbers sold as "safe", "non-additive", and containing "no chemicals" in a Beijing supermarket. (Photo: Michael Fabinyi)

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[4] PNG's national fisheries management plans are available on the Internet (http://www.fisheries.gov.pg/PolicyandRegulation/ManagementPlans/tabid/87/Default.aspx 2013). At the time of writing the new Management Plan for the sea cucumber fishery was not yet available on the webpage.

where staff are available to implement and enforce policies. The point of export is far more governable than the fishery under prevalent conditions in PNG, so it makes sense to concentrate management efforts on the exporting node of the chain.

The success of the revised Management Plan, however, rests on ensuring fishery closures are enforced effectively and at the right times. In the past, the monitoring of catches via monthly export reports was not timely enough, which allowed the fishery to overshoot the intended limits and render the whole plan ineffective. The key point for strengthening the total allowable catch (TAC) and closed season instruments are to make sure TACs are set at the appropriate levels for each province,– which will require ongoing stock assessments – and to ensuring TACs are monitored in "real time" and fishing stops when the TAC is reached. More broadly, a central, ongoing challenge for the governance of the beche-de-mer fish chain in PNG will be how to ensure the sustainability of the fishery in the context of the strong need for cash in villages, and strong market demand. Even though the revised Management Plan is a good option to the operational reality of the business, it may still be unequal to these demand- and supply-side pressures.

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Sea cucumber fisheries in Rasa Island Wildlife Sanctuary, Narra, Palawan, Philippines

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Joseph R. Paduga[1] and Rodulf Anthony T. Balisco[1]

Abstract

Sea cucumber fishing in Rasa Island Wildlife Sanctuary is an important source of livelihood among the coastal inhabitants of Narra Municipality, Palawan, Philippines. Sea cucumbers, however, are becoming overly exploited in most of their distribution range. Therefore, there is a need to determine the status of this fishery resource within the island to serve as basis for management interventions. Of the 24 species identified within the sanctuary, 3 species (*Actinopyga echinites, Holothuria scabra* and *Stichopus herrmanni*) are listed as threatened by the International Union for Conservation of Nature. Only 8 species were encountered at the intertidal and shallow subtidal reefs, and only 20 species were identified by the respondents. The current number of species in the sanctuary represents about 69% of the 35 reported number of commercially important sea cucumber species in Palawan. The survey for abundance and catch-per-unit-effort in five stations within the sanctuary revealed an average density of 52.95 ind. ha^{-1}. With an average catch-per-unit-effort of 1.79 kg person^{-1} h^{-1} and an average fishing time of 5 h, fishers could at least collect 8.95 kg per operation. The projected monthly earnings (PHP 1,600.00)[2] from sea cucumber fishing is much lower than what is claimed (PHP 3,937.50–5,571.00) by the respondents whose fishing activities covered a much wider area and depth.

Introduction

The Philippines is one of the largest producers of sea cucumbers, and represents a multi-million dollar fishery industry for the country (Akamine 2001). Statistics reveal that the annual volume of harvest in the Philippines reached its peak between 1985 and 1993, with an annual average of 3,478.89 t. However, the trend dropped towards 1998, and between 1998 and 2014, average annual production did not exceed 1,000 t (FAO 2016). Such decline was coupled with a dramatic increase in the number of exploited species, with only 26 species exploited in 2000 (Schoppe 2000) and 35 species in recent years, with the inclusion of more low-valued species (Jontila et al. 2014a).

If marine protected areas (MPAs) were established they could help promote the conservation of sea cucumbers in the Philippines (see Cabral et al. 2014; Horigue et al. 2014; Muallil et al. 2015). Sea cucumbers and other marine organisms were found to have recovered in effectively managed MPAs (Dumas et al. 2010; Dolorosa 2015), but species and biomass recovery is not possible in partially protected areas (Shears et al. 2006; Bobiles et al. 2016). Rasa Island Wildlife Sanctuary (RIWS) is an example of a partially protected MPA in the country. While the island has been declared as a protected area pursuant to RA 7586 or the National Integrated Protected Areas System Act of 1991, and now known as Rasa Island Wildlife Sanctuary through Presidential Proclamation 1000 series of 2006 (Widmann et al. 2010); however, only the terrestrial part of the island is fully protected. Its surrounding reefs remain open for many types of fishing activities, and sea cucumbers are among the commonly harvested species in RIWS but information about them is lacking.

The unregulated harvesting of sea cucumbers may affect both the diversity of these species and those people who are directly dependent on these resources. Thus, this study was conducted to determine the species diversity, abundance and sizes of sea cucumbers, catch-per-unit-effort and earnings of sea cucumber collectors in RIWS. These data are expected to serve as a basis in formulating local policies affecting the sustainable use of this fishery resource in RIWS.

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[2] PHP 1.00 = USD 0.02 (as of January 2016)

Materials and methods

Study site

Rasa Island Wildlife Sanctuary (9°13′21.25″N and 118°26′38.06″E) is a nearshore island located in Narra, Palawan, Philippines. The area of the island's shallow coast, coralline rocks and coral reefs is about 381.48 ha (Fig. 1).

Collection and identification of specimens

Between 4 April and 5 May 2015, samples were obtained from sea cucumber fishers operating in the vicinity of RIWS. Photos of sea cucumbers taken from RIWS between 2009 and 2010 were also examined. Identification was based on the works of Schoppe (2000), Kerr et al. (2006), Purcell et al. (2012) and Jontila et al. (2014a).

Abundance and catch-per-unit-effort

Sampling was timed with good weather and during low tide at night (19:00 to midnight) when the water was clear and free of ripples. Five sampling stations (Fig. 1) were established with two areas at each station: the Cortido Area (CA, or *Holothuria scabra* area) is located adjacent to the outer edge of the mangrove forest, and the Hanginan Area (HA, or *Stichopus* spp. area) is found close to the reef crest. In each area, several transects were surveyed to record the abundance of sea cucumbers and CPUE of a collector. Sea cucumber harvesting was carried out by a fisherman using a wooden pole (armed with a pointed metal piece on the tip) while standing in the bow of a *banca* (a traditional boat with an outrigger). The beginning and end of each transect were recorded with a global positioning system to estimate the distance covered in each 10-minute survey. The area of each transect was obtained as the product of the estimated length and approximate 5 m width of each transect (Table 1).

The abundance of sea cucumbers was calculated as the quotient of the number of individuals harvested per area for each transect (ind. ha^{-1}). CPUE was calculated using the data on catch and time spent fishing. Data among stations were compared using analysis of variance.

Assessment of fisher's catch and earnings

In total, 23 sea cucumber fishers (10 from Barangay Malinao and 13 from Barangay Antipuluan) from two nearby *barangays* (villages) were interviewed using a questionnaire. Interviews were

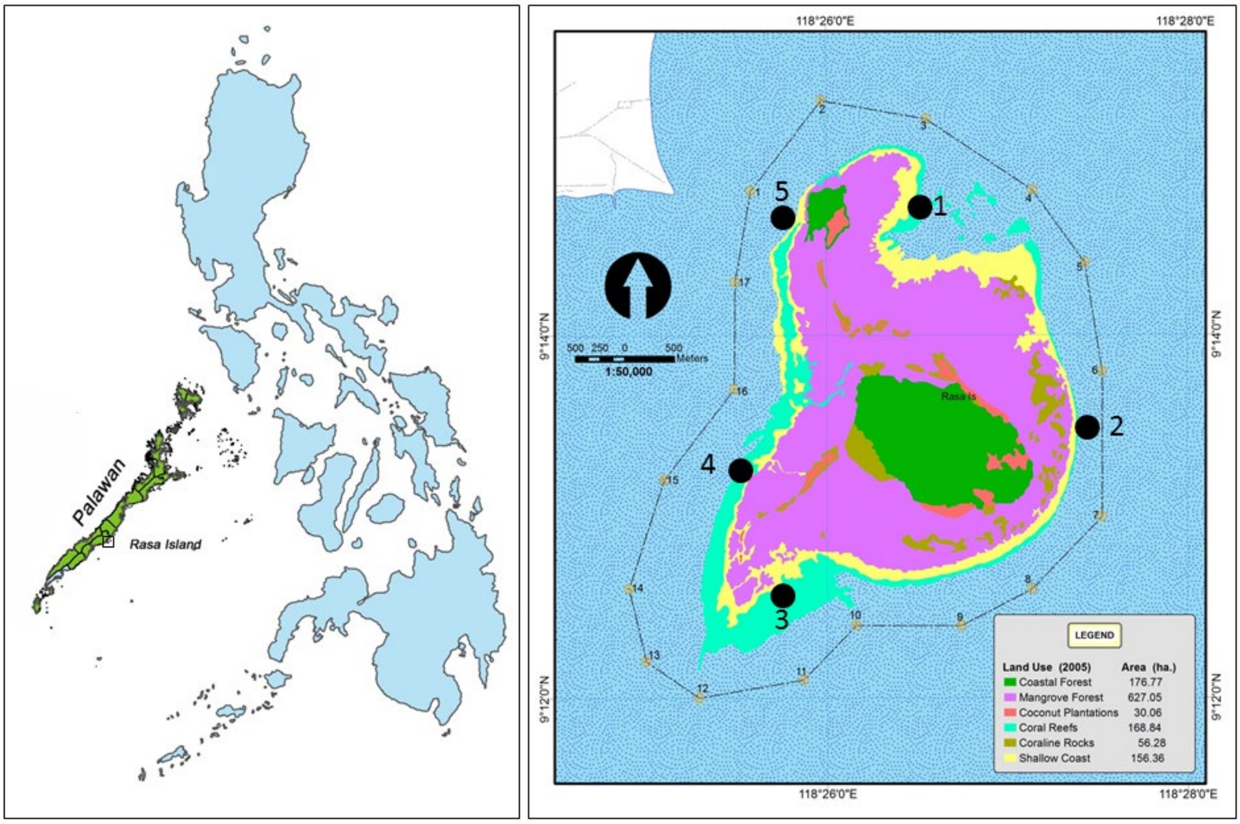

Figure 1. Location of Rasa Island Wildlife Sanctuary (RIWS) in Palawan (left) and the locations of five surveyed stations (right). The numbered lines surrounding the island indicate the boundaries as stipulated in the Presidential Proclamation 1000 series of 2006. Total area of the sanctuary is 1,980 ha (this includes the marked boundaries in the water as defined by the connected lines surrounding the island).

Table 1. Number of transects and area (m^2) covered at each station.

Station	Cortido Area		Hanginan Area		Total	
	Number of transects	Total area covered (m^2)	Number of transects	Total area covered (m^2)	Number of transects	Total area covered (m^2)
1	9	954.6	5	248.3	14	1202.9
2	4	542.1	5	551.9	9	1094.0
3	8	1231.1	9	925.7	17	2156.8
4	4	385.3	4	1021.1	8	1406.4
5	8	1440.8	6	342.3	14	1783.1
Total	33	4553.9	29	3089.3	62	7643.2

conducted to elucidate the status and trends of sea cucumber fisheries in RIWS. Most respondents belonged to the indigenous community and had an elementary educational background. Photos of live sea cucumbers were shown to respondents to identify the species involved in the fishery. The sizes of dried sea cucumbers processed by the respondents were also recorded.

Results

Species composition

In total, 24 commercially important species belonging to families Holothuriidae and Stichopodidae were recorded. Holothuriidae had the most number of species (20), while only 4 species belonged to the family Stichopodidae (Figs 2–4; Table 2). Only 8 of these 24 species were encountered during the survey while 20 species were identified by the respondents through photos (Table 2).

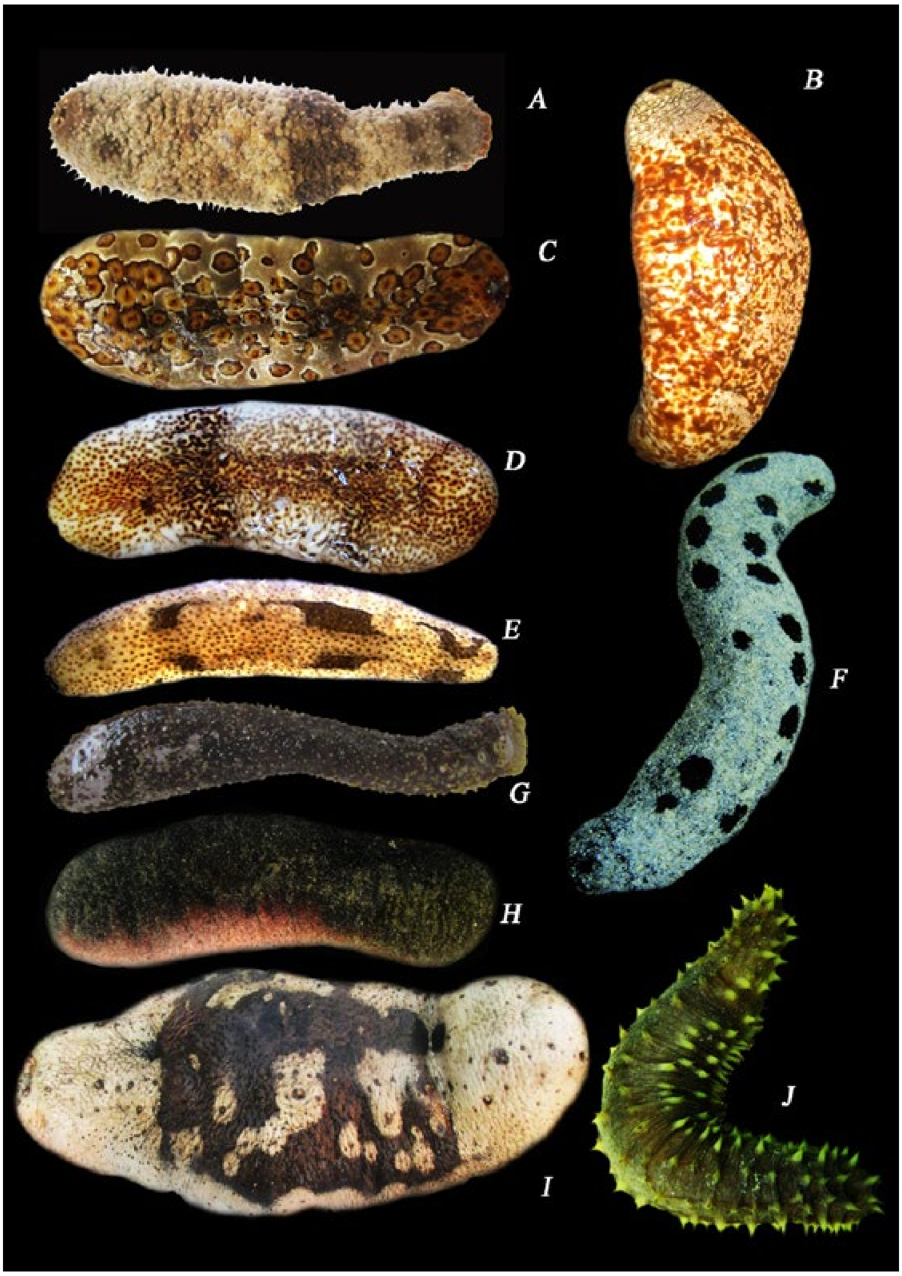

Figure 2. Family Holothuriidae: A) *Actinopyga echinites*, B) *A. lecanora*, C) *Bohadschia argus*, D) *B. vitiensis*, E) *B. marmorata*, F) *Holothuria atra*, G) *H. coluber*, H) *H. edulis*, I) *H. fuscogilva* and J) *H. hilla*.

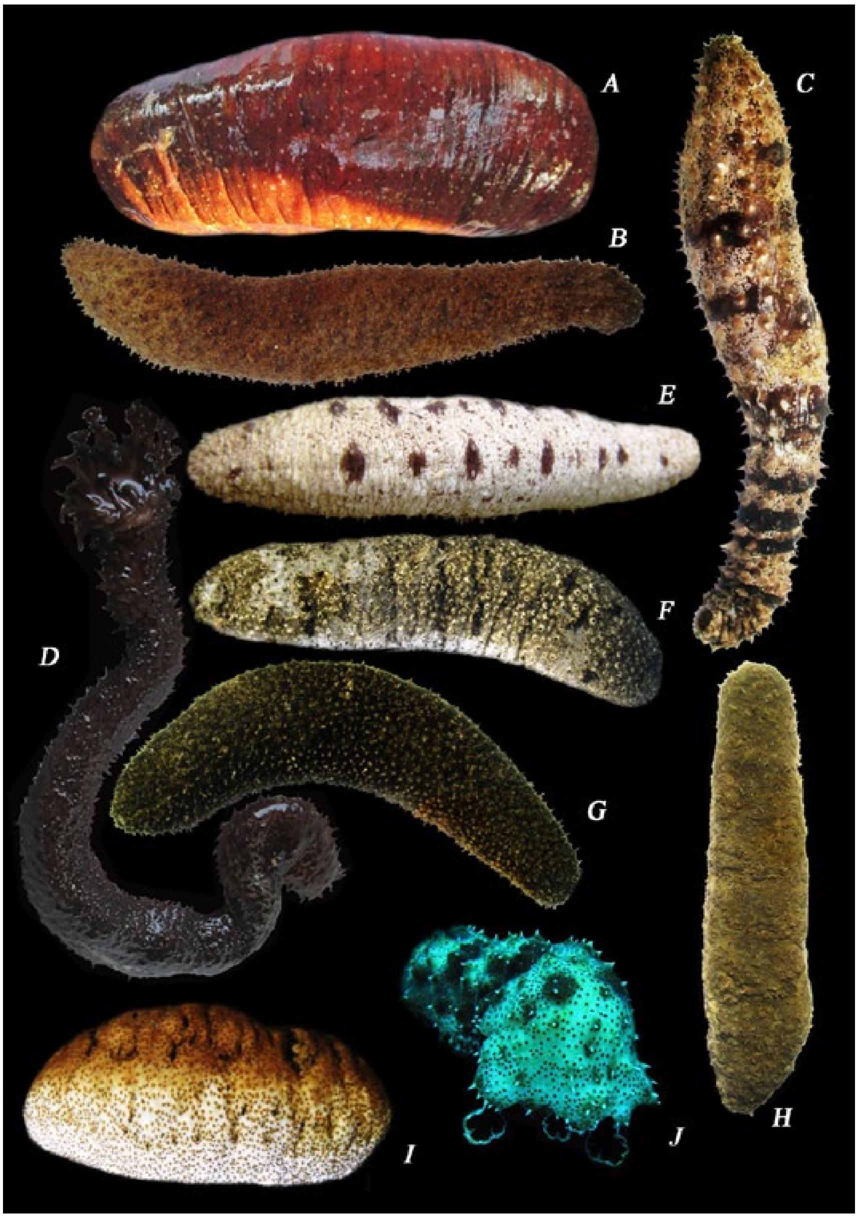

Figure 3. Family Holothuriidae: A) *Holothuria* aff. *edulis*, B) *H. fuscocinerea*, C) *H. impatiens*, D) *H. leucospilota*, E) *H. rigida*, F) *H. scabra*, G) *H.* sp. 1, H) *H.* sp. 2, I) *H. fuscopunctata* and J) *Pearsonothuria graeffei*.

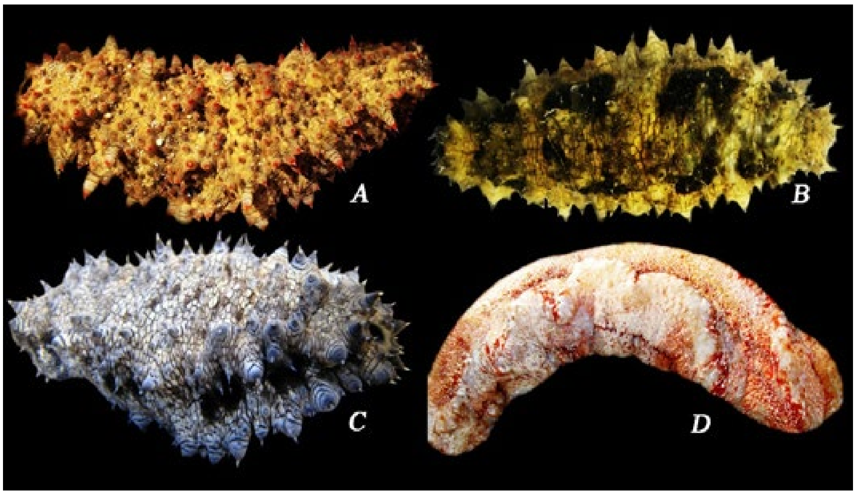

Figure 4. Family Stichopodidae: A) *Stichopus herrmanni*, B) *S. horrens*, C) *S. monotuberculatus* and D) *Thelenota anax*.

Table 2. List of commercially important sea cucumbers that are exploited in Rasa Island Wildlife Sanctuary, Narra, Palawan.

Scientific name	English name	Local name	Cortido and Hanginan areas (CA and HA)	Fishery dependent (interview)
Family Holothuriidae				
Actinopyga echinites	Deep-water redfish	Khaki / brown beauty	+	+
A. lecanora	White-bottomed sea cucumber / stonefish	Buli / monang		+
Bohadschia argus	Leopardfish	Matang-itik		+
B. vitiensis	Speckled sea cucumber / brown sandfish			
B. marmorata	Chalky sea cucumber		+	+
Holothuria atra	Lollyfish	Black beauty	+	+
H. coluber	Snakefish	Patola white		+
H. edulis	Pinkfish	Red beauty		+
H. aff. edulis	Pink sea cucumber	Lipstick		+
H. fuscogilva	White teatfish	Susuhan		+
H. fuscopunctata	Elephant trunkfish	Sapatos	+	
H. fuscocinerea	Ashen sea cucumber	Dagtaan		+
H. hilla	Tiger-tail sea cucumber	Bat-tuli		
H. impatiens	Bottleneck sea cucumber			+
H. leucospilota	White threadfish	Lawayan		+
H. rigida	Rigid sea cucumber			+
H. scabra	Sandfish	Curtido	+	+
Holothuria sp.1			+	+
Holothuria sp.2		Taba-taba	+	+
Pearsonothuria graeffei	Blackspotted sea cucumber	Pinya-pinya / mani-mani		+
Family Stichopodidae				
Stichopus herrmanni	Curryfish	Hanginan		+
S. horrens	Selenka's sea cucumber	Hanginan	+	+
S. monotuberculatus	Selenka's sea cucumber	Hanginan		
Thelenota anax	Amberfish	Legs		+
Total: 24			8	20

+ means "present"

Abundance and catch-per-unit-effort

The overall density of sea cucumbers in both CA and RA was 52.95 ind. ha^{-1}; the average density in CA and HA were 26.8 and 89.05 ind. ha^{-1}, respectively. Station 5 had the highest density (136.2 ind. ha^{-1}) followed by Stations 1, 3, 4 and 2 (Fig. 5).

Abundance in each area per station suggests that HA has the higher density of sea cucumbers than CA but these differences were not statistically significant ($p>0.05$).

Average CPUE was relatively similar in all stations, with an overall average of 1.79 kg person^{-1} h^{-1}. Stations 1 and 5 had the highest CPUE, while Station 2 had the lowest. The large confidence interval (error bars) suggest large variations of catch among transects (Fig. 6). Therefore, the CPUE across the five stations was not significantly different (p>0.05).

Assessment of fishers' catches and earnings

The historical volume of harvested sea cucumbers from RIWS reveals a declining pattern as perceived by respondents. In the past, harvests could be as

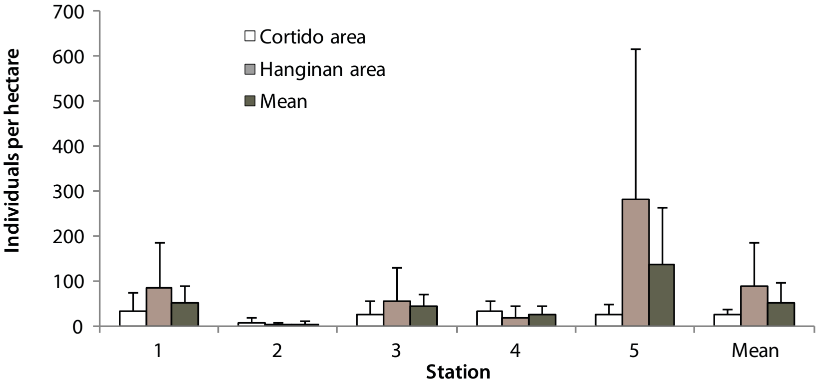

Figure 5. Average (±sd) density (ind. ha^{-1}) of commercially important sea cucumber species in Cortido and Hanginan Areas in Rasa Island Wildlife Sanctuary, Narra, Palawan.

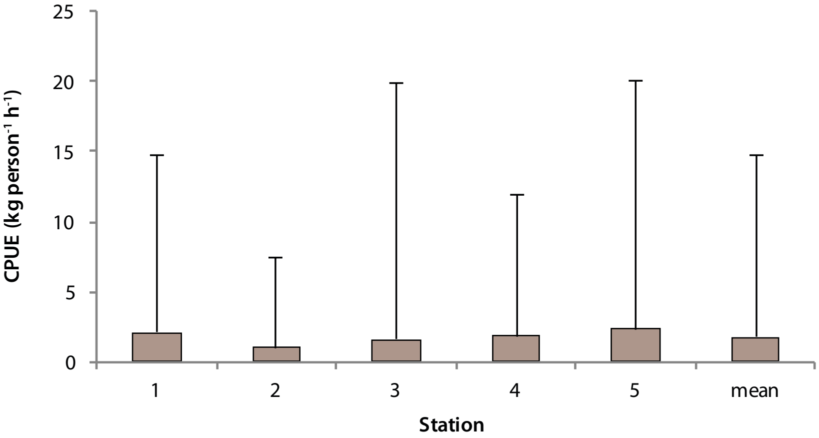

Figure 6. Average (±95% CI) sea cucumbers catch per unit effort (kg person^{-1} h^{-1}) in Rasa Island Wildlife Sanctuary, Narra, Palawan.

much as 120 kg per operation compared with only about 3 kg per operation currently (Fig. 7). Recently, respondents claimed that, on average, they can only process about 11.5 kg (±10.4; range: 2–30 kg) of fresh sea cucumber per week.

Respondents also claimed that sea cucumbers in recent years are much smaller than they were in the past. Average lengths of 43 cm (±24) were encountered in the past, while sizes of only 19 cm (±8.8) have been found in recent years (Fig. 8). The perceived sizes per species are also much smaller than the reported maximum and common sizes (Fig. 9).

Monthly earnings of sea cucumber fishers

Sea cucumber fishing and processing were identified as either main or secondary sources of earnings for respondents. The average monthly earnings of respondents ranged between PHP 3,937.50 and PHP 5,571.43. The average earnings derived from sea cucumber collection (PHP 4,108.70) represent 50% of the monthly earnings of respondents because in addition to sea cucumber fishing, respondents also engage in fishing for other species, farming or any day job to supplement their earnings.

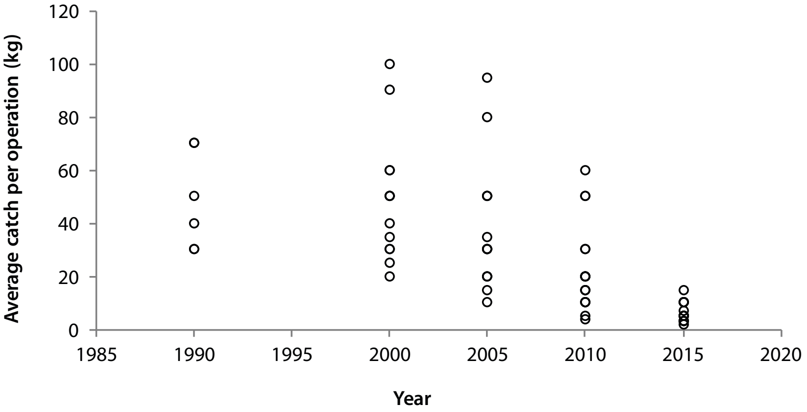

Figure 7. Historical volume (kg per fishing trip) of sea cucumbers harvested from Rasa Island, Narra, Palawan as perceived by respondents.

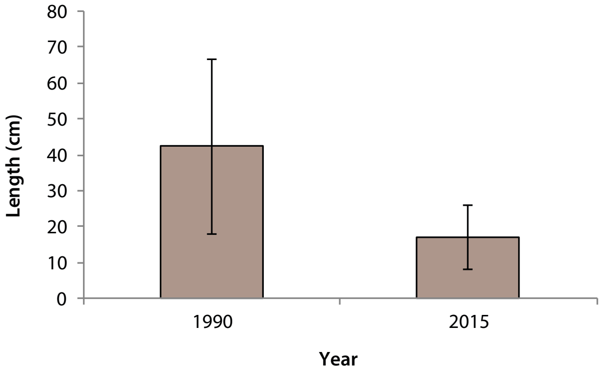

Figure 8. Average live length (±sd) of sea cucumbers fished in Rasa Island Wildlife Sanctuary in 1990 and 2015 as claimed by the respondents.

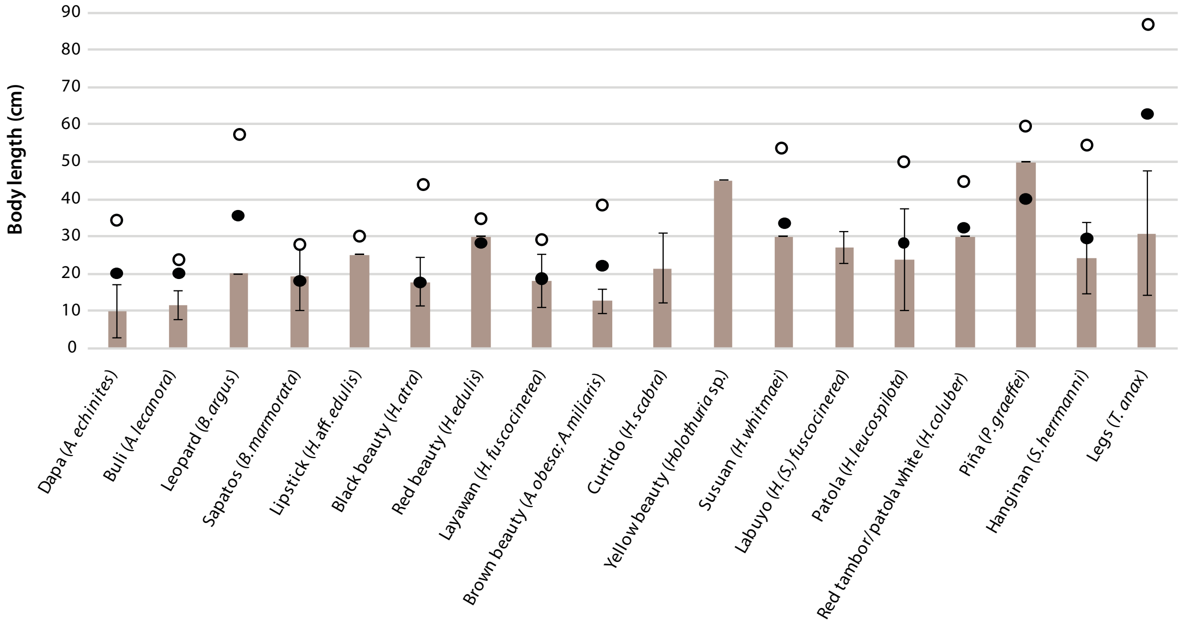

Figure 9. Current average body lengths (cm) of collected sea cucumbers as perceived by respondents. Unfilled circles represent the maximum sizes and and filled circles represent common sizes as reported by Purcell et al. (2012).

Discussion

Species composition

The number of commercially important sea cucumber species in RIWS is higher than in other areas of Palawan (Table 3). Such high numbers of species suggest that RIWS is an important sea cucumber habitat compared with other localities where only few species are recorded. The relatively high species richness around Rasa Island could be related to the varied types of habitat (i.e. extensive mangrove forest, sea grass meadows, reef flat and slope, and sandy habitats) around the island, which are suited for both young and mature sea cucumbers.

Most of the species recorded in this study were collected by fishers from shallow areas, except for *Actinopyga lecanora, Bohadschia marmorata, Holothuria* aff. *edulis, Pearsonothuria graeffei* and *Thelenota anax,* which were obtained in deeper parts of the reef. Some sea cucumbers inhabit areas down to 30 m depth (Purcell et al. 2012), therefore, additional species may be found with extensive survey at deeper parts of RIWS.

Jontila et al. (2014a) recorded 44 species of sea cucumbers in Palawan, but with the current findings in RIWS, 9 more species were added; therefore, at least 53 sea cucumber species are found in Palawan. This is higher than for other areas in

Table 3. Number of commercially and non-commercially important sea cucumber species in different parts of Palawan, Philippines.

Area	Commercially important	Non-commercially important	Total species	Author and year
Johnson Island, Roxas, Palawan	14	--	14	Paalan 2009
Aborlan (east coast), Palawan	6	2	8	Quillope 2011
Sta. Lucia, Puerto Princesa City, Palawan	10	2	12	Buri 2012
Brooke's Point, Palawan	13	12	25	Canonico 2013; Pitong 2013
Quezon, Palawan	8	3	11	Collantes 2013
Caruray, San Vicente, Palawan	16	9	25	Sangutan 2015
Tubbataha Reefs Natural Park, Palawan	16	2	18	Dolorosa 2015
Palawan	26	--	26	Schoppe 2000
Palawan	35	9	44	Jontila et al. 2014a
Rasa Island, Narra, Palawan	24	Not documented	24	This study

the Philippines, and in other countries. For example, Kerr et al. (2006) recorded 49 holothuroid species in Central Visayas (Negros, Cebu, Bohol and neighbouring localities), 35 species were found in Bolinao-Anda, Pangasinan (Olavides et al. 2010), 18 species in the South China Sea (Dongsha Island, Namsha Island, Xisha Island and Zongsha Island) (Xiangmin 2004), 21 species in Papua New Guinea (Polon 2004), and 41 species in northwestern Australia (Ashmore Reef, Cartier Reef and Mermaid Reef) (Smith et al. 2002; Rees et al. 2003).

Among the 24 species of sea cucumbers found near Rasa Island, 3 species are listed as threatened (IUCN 2015; Conand et al. 2014). *Holothuria scabra* (Holothuriidae) is listed as "endangered" or "at high risk of extinction", while both *Actinopyga echinites* (Holothuriidae) and *Stichopus herrmanni* (Stichopodidae) are listed as "vulnerable" or "at risk of extinction". Globally, Conand et al. (2014) reported seven species of sea cucumbers already listed as "endangered" and nine species as "vulnerable". At the local level, however, more species are likely threatened because the sizes of dried sea cucumbers collected from RIWS fall below the size limits set in neighbouring countries (Purcell et al. 2012).

Some of the exploited species (*Holothuria marmorata, H. vitiensis, H. coluber, H. rigida, H. scabra, H. lineata, Acaudina* sp., *Stichopus monotuberculatus, S. ocellatus, S. quadrifasciatus, S. rubermaculosus, S.* aff. *rubermaculosus* and *S. vastus*) recorded in this study and by Jontila et al. (2014a) were not recorded by Akamine (2005). This suggests that some species have already been overharvested, and attention has shifted to previously unexploited species. There is a paradigm shift in terms of the commercial value of sea cucumbers. Because most of the high-value species are overharvested, traders are now turning medium-value species into high value. In recent years, some species with no commercial value before now turned into low-value species, possibly to sustain the demand in beche-de-mer in the international market.

Sea cucumbers are prone to overharvesting (Hasan 2005; Kerr 2006; Purcell et al. 2012; Conand et al. 2014); thus, a management plan to protect and rebuild populations of these species and their habitat is important in order to avoid the collapse of the fishery. Long-term effective protection of marine parks could allow the recovery of marine species as noted in the Tubbataha Reefs Natural Park (TMO 2013; Dolorosa 2015). In New Zealand, long-term protection (for nearly 30 years) of marine parks has resulted in an increase of the legal size of lobsters, which are 11 times more abundant and represent 25 times more biomass in the no-take marine park following park establishment, while no significant change has occurred in lobster abundance in partially protected marine parks (Shears et al. 2006). Thus, for the local government to revive the lost sea cucumber populations in RIWS, a long-term effective fishing closure in needed in specific areas.

Abundance and catch-per-unit-effort

Among the stations surveyed, Station 5 may be the least visited by fishermen because of its narrow reef and proximate distance from the mainland (S. Villalva pers. comm.). Such low fishing pressure at Station 5 could be one of the reasons for the high abundance of sea cucumbers at that station compared with other stations. Abundance was low at Station 4 because it is close to the docking point of the island rangers who also engage in sea cucumber fishing. These findings conform to the principle of establishing a marine sanctuary where organisms have a chance to recover in an area spared from fishing pressure (Lane 2008; Toral-Granda et al. 2008; Dumas et al. 2010; Olivades et al. 2010; Dolorosa 2015).

The overall density of sea cucumbers in RIWS (52.95 ind. ha^{-1}) was higher than in Tubbataha Reefs Natural Park (TRNP), but lower compared with other areas in Palawan and elsewhere (Table 4). Also, the abundance of *H. scabra* and *S. horrens* in RIWS was far lower than in other areas (Table 5). The observed sizes in RIWS, however, were far smaller than those in TRNP (Dolorosa 2015) due to unregulated harvesting.

The CPUE in this study (8.97 kg person^{-1} operation^{-1} or 122 ind. person^{-1} operation^{-1}) was comparable with the CPUE in other areas. Rasolofonirina et al. (2004) reported a CPUE of 4.96 kg to 10.67 kg fisher^{-1} day^{-1} (corresponding to 16.5 to 35.57 ind. fisher^{-1} day^{-1}) in Toliara, southwest Madagascar. In Cuba, CPUE is about 1,200 ind. boat^{-1} day^{-1} (Alfonso et al. 2004).

Monthly earnings of sea cucumber fishers

The dwindling volume of commercially important sea cucumbers as perceived by respondents has been also reported in other parts of the Philippines and other countries such as Malaysia and Egypt (Choo 2008; Purcell et al. 2012). In Mahout Bay, Sultanate of Oman, the mean sizes of sea cucumbers were much smaller in areas close to human populations. The reported average size of sea cucumbers in the Sultanate of Oman ranges between 16.6 cm and 26.8 cm (Al-Rashdi et al. 2007), which is comparable with the recent perceived sizes of sea cucumbers in RIWS.

Dried lengths of harvested sea cucumbers (see Fig. 10 on p. 18) from RIWS were generally categorised as small on the local market and are smaller than the size limit imposed in other countries (see

Table 4. Average density (ind. ha^{-1}) of sea cucumbers in Rasa Island Wildlife Sanctuary and in other areas.

Locality	No. of species	Density (ind. ha^{-1})	Source
Australia (Mermaid Reef) (*Thelenota ananas*)	1	8.82	Rees et al. 2003
Panama (*Holothuria mexicana*)	1	161.80	Guzman and Guevara 2002
Panama (*Isostichopus badionotus*)	1	117.40	
Panama (*Actinopyga multifidus*)	1	4.90	
NorthWestern Australia (Ashmore and Cartier Reef) (*Holothuria whitmaei*)	1	1.0	Shiell 2004
Western Australia (Rowley Shoals) (*H. whitmaei*)	1	9.1	
Western Australia (Ningaloo Reef) (*H. whitmaei*)	1	19.3–27.2	
North Western Australia (Mermaid Reef and Coral Bay) (*H. whitmaei*)	1	19.3–27.2	
Bolinao-Anda Reefs, Pangasinan	35	63.00	Olivades et al. 2010
Caruray, San Vicente, Palawan	25	618.00	Sangutan 2015
Tubbataha Reefs, Palawan	8	41.93	Dolorosa 2015
New Cuyo and Malcampo, Roxas, Palawan	4	66.50	Sornito 2015
Ilocos Sur	14	700.00	Sanidad and Sanidad 2015
Rasa Island (Cortido Area, CA)	5	26.80	This study
Rasa Island (Hanginan Area, HA)	7	89.05	This study
Rasa Island (combined CA and HA)	8	52.95	This study

Table 5. Density (ind. ha^{-1}) of *H. scabra* and *Stichopus* spp. in Rasa Island Wildlife Sanctuary, Narra, Palawan compared with other areas.

Species	Locality	Density (ind. ha^{-1})	Source
Holothuria scabra	Rasa Island, Narra, Palawan	19	This study
	Mahout Bay, Sultanate of Oman	1770–4000	Al-Rashdi et al. 2007
	Solomon Islands	20–220	Mercier et al. 2000
	Warrior Reef, Torres Strait, Australia	472.83 (1995/96)	Skewes et al. 2000
		102.04 (1998)	
		137.76 (2000)	
	Abu Rhamada Island, Red Sea	(before fishing): 8570–9519	Hasan 2005
		(after 4 years of fishing): 40–3070	
Stichopus horrens	Rasa Island, Narra, Palawan	25	This study
	Western Central Pacific	70	Carpenter and Niem 1998
	Santa Cruz, Galapagos	>100 (ind. 100 m^{-2})	Hearn and Pinillos 2006

Purcell et al. 2012). Five of the harvested species in RIWS have average dried sizes falling within and below the country's 5-cm size limit across species (DA 2013). Sizes at sexual maturity vary among species (Purcell et al. 2012) and the implementation of the country's policy on size limit might be only beneficial for species maturing at small sizes. In the study by Kinch et al. (2008) and Hasan (2005), the declining population status of sea cucumbers was attributed to high fishing pressure. At Helen Reef, Republic of Palau, populations of trochus and giant clams are extremely depleted due to intense harvesting (Weng and Guilbeaux 2000). Similarly, trochus populations in TRNP have sharply declined after some poaching events (Jontila et al. 2014b; Dolorosa et al. 2016).

Population declines of sea cucumber can be a consequence of overfishing (Hasan 2005; Anderson et al. 2011; Kumar 2012). Regional assessments of sea cucumber fisheries made by Anderson et al. (2011) revealed that population declines from overfishing

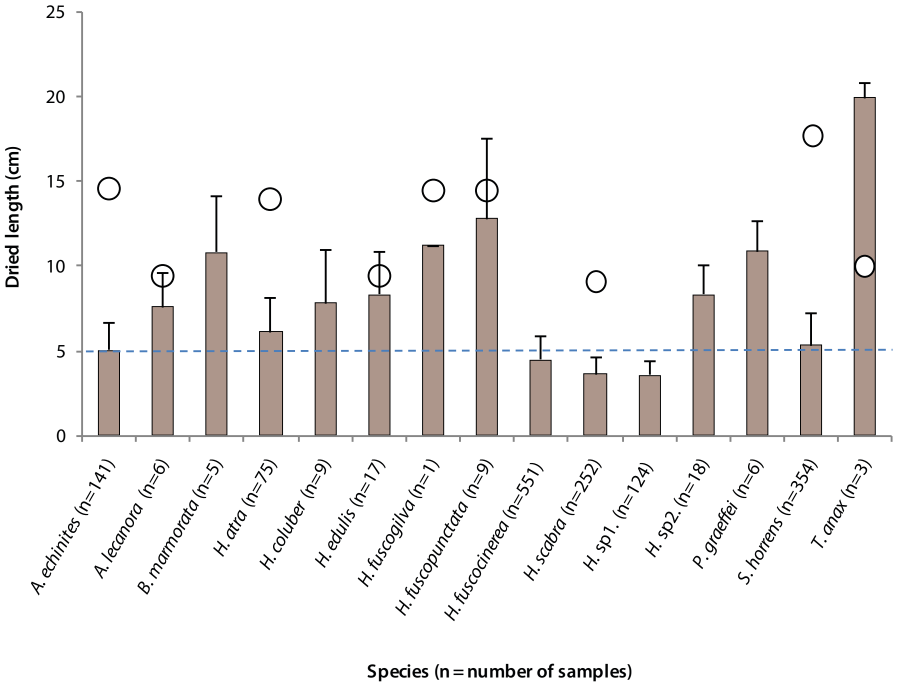

Figure 10. Average (±SD) dried body length of sea cucumbers harvested in Rasa Island Wildlife Sanctuary. Open circle represents the dried size limit imposed in other countries as reported by Purcell et al. (2012). Horizontal broken line indicates the size limit in the Philippines as stipulated in BFAR Administrative Circular No. 248 (DA 2013).

occurred in 81% of sea cucumber fisheries. About 51% of harvesters have moved from nearshore to offshore areas, and 76% of harvesters have shifted collecting high-value species to low-value species, and the average harvested sea cucumber body size has declined by 35%. All of these factors are considered to be signs of overexploitation.

Figure 10. Average (±sd) dried body length of sea cucumbers harvested in Rasa Island Wildlife Sanctuary. Open circle represents the dried size limit imposed in other countries as reported by Purcell et al. (2012). Horizontal broken line indicates the size limit in the Philippines as stipulated in BFAR Administrative Circular No. 248 (DA 2013).

The average monthly earnings by interviewed sea cucumber fishers was twice as high as the projected earnings of sea cucumber fishers at CA and HA (PHP 1,600.00), but comparable to the monthly earnings of sea cucumber fishers from northern Luzon (PHP 3,000–5,000) (Sanidad and Sanidad 2015). The study shows that RIWS is an important habitat for various species of sea cucumbers. However, abundance of sea cucumbers in the RIWS is very low compared to other areas. The respondents declared a decline in both volume of catch and sizes of sea cucumbers collected from RIWS. The catch per unit effort is low but earnings derived from processing constitute at least 50% of the monthly earnings of the respondents. Given the significant contribution of sea cucumber fisheries in the lives of the respondents, it is important to sustainably manage and prevent the collapse of this fishery resource. Other than the country's uniform dried size limit, species-specific live size limits must be determined and implemented to prevent the capture of immature species and enhance the earnings derived from sea cucumber fishing. Strict implementation of laws specific to RIWS is needed to allow the recovery of sea cucumber stocks.

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Species composition, density and distribution of sea cucumbers (Holothuroidea) at Arreceffi Island, Honda Bay, Palawan, Philippines

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Abstract

This study was conducted to determine the species composition, distribution, density and size structure of sea cucumbers at Arreceffi Island in Honda Bay, Palawan, Philippines. Three stations covering different habitats (intertidal flats, seagrass beds, and coral reefs) were surveyed during the day and night by walking, snorkeling and scuba diving. In total, 15 species in the families Holothuriidae (11 species), Stichopodidae (3 species) and Synaptidae (1 species) were recorded. Some rare *Stichopus* and high-value species were recorded along with other aggregating species of the family Holothuriidae. Density generally ranged from 0.3 to 19.0 ind. 100 m^{-2}, and greatly varied depending on habitat.

Introduction

Sea cucumbers are among the heavily exploited invertebrates in the Philippines although their collection is unregulated due to poor implementation of pertinent laws. Information on sea cucumber populations is also very limited. Most of the earlier works focused on taxonomy and species inventories (Domantay 1934, 1960; Reyes-Leonardo 1984; Tan Tiu 1981; Schoppe 2000a; Kerr et al 2006). In recent years, sea cucumber gatherers have observed that catches are declining, and market trends also suggest this (Akamine 2005; Choo 2008b; Brown et al. 2010). In fact, since the Philippines' production peaked in the 1980s, the supply has not recovered and has shifted from a high-value and low volume focus, to a high-volume and low-value species one (Akamine 2002, 2005). Although artisanal in nature, the sea cucumber fishery has provided substantial income to meager fishermen across the country (Labe 2009). In 2012, dried sea cucumbers ranked tenth among the fishery commodities of the Philippines in terms of export value: 149 metric tons (mt) amounting to USD 1,849,230 (BFAR 2014). Despite declining production over the years (Akamine 2005), the country remains among the top suppliers of sea cucumbers in Asia (Choo 2008a), and the province of Palawan is one of its major producers (Brown et al. 2010).

Recognizing the economic and ecological importance of sea cucumbers, the Bureau of Fisheries and Aquatic Resources (BFAR) issued an Administrative Circular No. 248 in 2013, which imposed size limits and required permits for those people engaged in the sea cucumber trade. However, its implementation is still a challenge and sea cucumber gathering remains unregulated. Initial assessments in Palawan have revealed that areas open to harvesting have very little populations remaining, or populations have been depleted (Jontila et al. 2013). High-value species were seldom encountered in shallow sites of Bataraza, Quezon, El Nido and Roxas in Palawan except around Arreceffi Island in Honda Bay, Puerto Princesa City, where viable populations were noted (Jontila et al. 2014).

Sea cucumber populations are difficult to revive once they are depleted due to their limited mobility, late maturity, density-dependent reproduction, and low rates of recruitment (Uthicke and Benzie 2000; Uthicke 2004; Bruckner 2005). It is, therefore, very important to identify and protect the areas where they remain abundant. This study was therefore conducted to provide an initial assessment on the status of sea cucumber in Arreceffi Island in terms of species composition, abundance and distribution.

Methods

Study site

Arreceffi Island, also known as Dos Palmas Island Resort and Spa, is situated in the middle of Honda Bay in Palawan, Philippines. The island has thick mangrove cover, seagrass beds and coral reefs that are nearly pristine (Fig. 1). Although open to tourism, extraction of any resources is not allowed within its vicinity. The island's "no-take policy" has allowed its marine resources to flourish naturally, and sea cucumbers are abundant. They are widely distributed around the island from shallow seagrass beds, mangroves, coralline flats and coral reefs.

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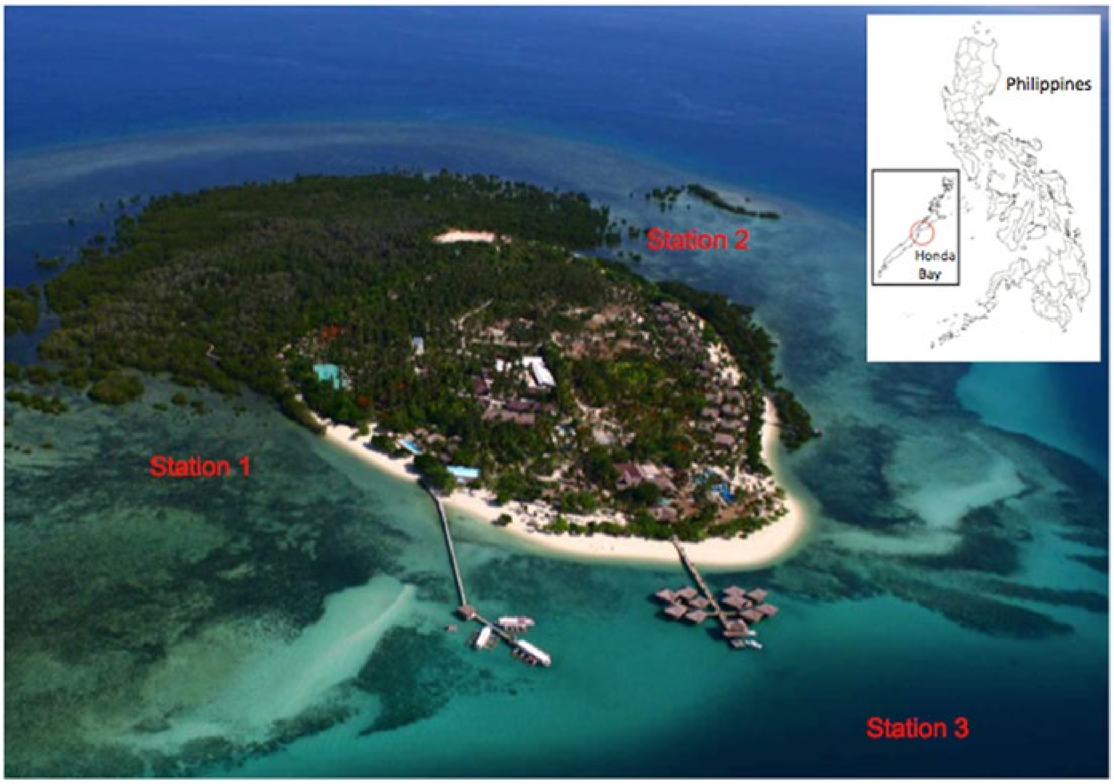

Figure 1. Aerial photograph of Arreceffi Island in Honda Bay, Palawan, Philippines showing the study's stations.

Three stations, representing different habitats, were established around the island (Fig. 1). Station 1 was mainly covered by seagrasses *Cymodocea, Halophila, Halodule, Thalassia and Enhalus.* The substrates ranged from sandy to silty. Station 2 was also covered with seagrasses but not as much as in Station 1. Its substrates were generally silty to sandy, with portions of coralline flats having patches of sand and rubble. One transect in this station was near the mangrove forest dominated by Rhizophora stylosa. Station 3 is a coral reef area, ranging in depth range from 5 to 8 m. Foreshore reef areas have mixed live corals, dead coral with algae, and sandy bottom, with little rubble. Few boulders of massive corals Porites were also present at this site.

Species identification, distribution, density and size structure

At each station, three transects measuring 50 m x 2 m were laid perpendicular to the shore at 10–15 m apart. Day and night surveys were conducted except at Station 3 where only night surveys were carried out. Snorkeling and wading were done in shallow areas while the coral reefs of Station 3 were assessed through scuba diving. Also, a timed search was done at Station 3 instead of a belt transect. This search was standardized (10 mins ± 4 mins) to cover almost the same area (100 m^2) with that of the transects surveyed through snorkeling or wading.

All sea cucumbers encountered along the transects and during the timed search were identified, counted and measured (cm), following their body contour using a bendable ruler. Care was taken during measurement so as not to touch the body to avoid constriction. In shallow areas, cryptic and burrowing species were pulled out and allowed to relax in situ before taking their measurements. A sample of each species was collected and immersed in 5% solution $MgCl_2$ for around 10 minutes to relax the body and avoid evisceration during preservation. Measurements and photographs of the dorsal and ventral sides were taken after relaxation. Specimens were then preserved in either 95% ethanol or a 10% saline formalin solution. Species were identified based on their external anatomical features and an examination of spicules using the standard protocol. Identification was based on Conand (1998), Schoppe (2000a), Massin et al. (2002), Kerr et al. (2006), and Purcell et al. (2012).

Results

Species composition and distribution

In total, 15 species of sea cucumbers, belonging to the families Holothuriidae (11 species), Stichopodidae (3), and Synaptidae (1) were recorded in this study (Table 1). Holothuriids include the genera *Actinopyga* (1 species), *Bohadschia* (2), *Holothuria* (7),

Table 1. Species composition and distribution of sea cucumbers across study sites, and substrate and habitat type at Arreceffi Island, Honda Bay, Palawan, Philippines.

Stations:	1		2		3
	Seagrass beds	Coralline flats with seagrass	Seagrass beds	Coralline flats with seagrass	Coral reefs
Family Holothuriidae					
1 *Actinopyga lecanora*	+		+		
2 *Bohadschia marmorata*	+		+		
3 *B. vitiensis*	+				
4 *Holothuria leucospilota*	+	+	+	+	
5 *H. atra*	+	+	+	+	
6 *H. coluber*	+	+	+	+	
7 *H. edulis*				+	+
8 *H. fuscocinerea*	+				
9 *H. scabra*	+		+		
10 *Holothuria gracilis*					+
11 *Pearsonothuria graeffei*					+
Family Stichopodidae					
1 *Stichopus noctivagus*					+
2 *S. rubermaculosus*					+
3 *S. vastus*					+
Family Synaptidae					
1 *Synapta maculata*	+	+	+		
Total:	9	4	7	4	6

+ means "present"

and *Pearsonothuria* (1). The other two families were only represented by genera *Stichopus* and *Synapta.*

Most of the holothuriid species (8) were encountered at Station 1. This area was mainly covered with seagrasses but coralline flats and patches of sand as well as rubble were also dominant towards the station's seaward eend. Among the commonly encountered species in seagrass areas were *Actinopyga lecanora* (Fig. 2a), *Bohadschia marmorata* (Fig. 2b), *B. vitiensis* (Fig. 2c), *Holothuria atra* (Fig. 2d), *H. leucospilota* (Fig. 2h), *H. scabra* (Fig. 2i) and *Synapta maculata* (Fig. 2o). At night, *H. fuscocinerea* was also seen in the same area together with the species mentioned. In coralline flats and the sandy seagrass parts of this station, *H. atra* and *H. leucospilota* were also encountered along with *H. coluber* (Fig. 2e).

Individuals of *H. atra undergoing fission (Fig. 2d inset) were noted also in the* sandy seagrass areas of Station 1. Variants of some species were documented as well, such as yellow *B. marmorata* with irregular brown blotches on its dorsum (Fig. 2b, inset), and the black variant of *H. scabra,* formerly named as *H. scabra* var. *versicolor* (Fig. 2i). The same species at Station 1 were also noted at Station 2, except there were no *B. vitiensis* or *H. fuscocinerea.* There were also aggregations of *H. scabra* in silty-sandy areas near the stands of mangrove *Rhizopora stylosa.* On the other hand, all *Stichopus* species and three holothuriid species that were not seen in the previous stations were encountered at Station 3, which is a coral reef area. *Stichopus* included *S. noctivagus* (Fig. 2l), *S. rubermaculosus* (Fig. 2m) and *S. vastus* (Fig. 2n). Holothuriids included *H. edulis* (Fig. 2f), *H. gracilis* (Fig. 2j), and *Pearsonothuria graeffei* (Fig. 2k). *S. vastus* (Fig. 2n) was already noted in a previous survey of Arreceffi Island (Jontila et al. 2014) but a different variant was encountered in this survey. Within the reef area, *H. edulis* was found on sandy substrate with patches of live corals and rubble, while *P. graeffei* was spotted feeding on dead corals covered with algae.

Sea cucumber density

To account for both the burying and nocturnal species, day and night surveys were conducted at all stations, except Station 3 where only night surveys were carried out due to logistical constraints. Most of the species displayed varying densities across the stations between day and night sampling. During the day, *Synapta maculata* at Station 1 had the highest mean density (±SD)

Figure 2. Sea cucumber species at Arreceffi Island, Honda Bay, Palawan, Philippines.

Family Holothuriidae:

a) *Actinopyga lecanora*, b) *Bohadschia marmorata* (inset: yellow variant), c) *B. vitiensis*, d) *Holothuria atra* (inset: specimen just undergone fission), e) *H. coluber* (inset: ventral side of the mounth), f) *H. edulis* (dorsal and ventral sides), g) *H. fuscocineraea*, h) *H. leucospilota*, i) *H. scabra* (grey and black variants), j) *H. gracilis*, k) *Pearsonothuria graeffei* (inset: mouth with its tentacles);

Family Stichopodidae:

l) *Stichopus noctivagus*, m) *S. rubermaculosus*, n) *S. vastus*; Synaptidae: o) *Synapta maculata*.

at 14.0±10.5 ind. 100 m^{-2}. During the night, *Bohadschia marmorata* was quite abundant with an estimated density of 19.0±2.6 ind. 100 m^{-2} (Table 2). Few individuals of *H. fuscocinerea* (3.0±2.6 ind. 100 m^{-2}) were also noted at night, sharing the habitat with *B. marmorata. Holothuria atr*a was also abundant at Station 1, and its densities remained almost the same during the day (8.7±2.1 ind. 100 m^{-2}) and night (9.3±2.5 ind. 100 m^{-2}). *Actinopyga lecanora* and *B. vitiensis* also displayed the same densities during both surveys at Station 1. *Holothuria scabra* was found at both Stations 1 and 2, its density was highest during daytime at Station 2 at 9.3±7.1 ind. 100 m^{-2}. This could be due to sandy-silty substrate in Station 2 that is more preferred by this species (Mercier et al. 2000). In contrast, *Holothuria edulis, H. gracilis* and *Pearsonothuria graeffei* were only encountered at Station 3, with densities estimated at 8.7±4.7, 0.3±0.6 and 1.0±1.2 ind. 100 m^{-2}, respectively. It is also noted that *Stichopus noctivagus*, *S. rubermaculosus* and *S. vastus* were only noted at Station 3. These species, together with *P. graeffei*, are usually found in reef areas (Purcell et.al. 2012).

Size structure

Mean and maximum sizes of sea cucumbers encountered during the survey are presented in Table 3. Sizes were generally close to or within the reported range of measurement, except for few species. For instance, the mean length (±SD) for *H. coluber (*37.6±4.6) is more than twice as long than its reported mean size in the country (18 cm), but comparable to that recorded in Indonesia (26 cm), New Caledonia (40 cm) and Papua New Guinea (40 cm) (Purcell et al. 2012). Similarly, *S. noctivagus* measured 27 cm but Kerr et al. (2006) noted that this species grows to only about 20 cm. In Pulau Besar, Johore Marine Park in Malaysia, *S. rubermaculosus size ranges from* 26.0 cm to 28.5 cm, but a larger specimen measuring 34 cm was noted in this study. *Stichopus vastus mean (*49.0± 10.6 cm) and maximum sizes (56.0 cm) were also higher than its reported measurements at 34.0 cm and 35.0 cm, respectively (Purcell et al. 2012).

Discussion

The recorded number of sea cucumber species in this study represents 34% of the total species in Palawan (Jontila et al. 2014). Many were noted to have overlapping distribution, particularly the holothuriid species, which are known to inhabit shallow, sheltered lagoons and inner reef flats with silty to sandy substrates (Conand 1998; Jaquemet et.al. 1999) and sheltered coral reef

Table 2. Mean (±SD) density (ind. 100 m^{-2}) of sea cucumbers during day and night surveys at Arreceffi Island, Honda Bay, Palawan, Philippines.

		Day		Night		
		Station 1	Station 2	Station 1	Station 2	Station 3
Family Holothuriidae						
1	*Actinopyga lecanora*	4.0±1.0	0.3±0.6	4.0±2.0		
2	*Bohadschia marmorata*	1.7±1.5	0.7±1.2	19.0±2.6	2.7±1.5	
3	*B. vitiensis*	1.0±1.0		1.0±1.0		
4	*Holothuria leucospilota*	8.7±2.1	3.0±3.0	9.3±2.5	1.7±1.5	
5	*H. atra*	2.0±2.0	0.7±1.2	0.3±0.6		
6	*H. coluber*					8.7±4.7
7	*H. edulis*			3.0±2.6		
8	*H. fuscocinerea*	3.7±1.2	0.7±0.6	1.7±1.5	1.0±1.7	
9	*H. scabra*	2.7±2.5	9.3±7.1	1.7±2.9	2.0±2.0	
10	*H. gracilis*					0.3±0.6
11	*Pearsonothuria graeffei*					1.0±1.2
Family Stichopodidae						
1	*Stichopus noctivagus*					0.3±0.6
2	*S. rubermaculosus*					0.3±0.6
3	*S. vastus*					0.7±1.2
Family Synaptidae						
1	*Synapta maculata*	14.0±10.5	3.3±1.5	4.7±1.5	3.7±3.2	

* Spotted also during the day during the free dive survey around the island.

Table 3. Comparison of mean (± SD) length (cm) and maximum length of sea cucumbers recorded in this study and that of Purcell et al. (2012).

			This study		**Purcell et al. 2012**	
		n	Mean (SD) length (cm)	Maximum length (cm)	Mean/range length (cm)	Maximum length (cm)
Family Holothuriidae						
1	*Actinopyga lecanora*	24	20.1±3.3	25	20	24
2	*Bohadschia marmorata*	73	17.6±5.1	23	18	26
3	*B. vitiensis*	6	25.7±3.2	31	25–35	40
4	*Holothuria leucospilota*	62	17.7±5.5	29	26*/15–30	28/45
5	*H. atra*	5	37.6±4.6	43	18–40	60
6	*H. coluber*	26	25.7±6.4	39	20	38
7	*H. edulis*	9	27.9±4.4	32	20	30
8	*H. fuscocinerea*	21	31.9±7.8	41	37*/23–50	65/50
9	*H. scabra*	47	19.0±5.5	33	19–37	40
10	*Holothuria gracilis*	1	31.0	31		
11	*Pearsonothuria graeffei*	2	37.0±5.7	41	17–35	45
Family Stichopodidae						
1	*Stichopus noctivagus*	1	27.0	27	20**	20
2	*S. rubermaculosus*	1	34	34	26.0–28.5***	28.5
3	*S. vastus*	2	49.0± 10.6	56	33–35	35
Family Synaptidae						
1	*Synapta maculata*	77				

* Romero and Cabansag (2014)
** Kerr et al. (2006)
*** Massin et al. (2002)

edges with hard substrates (Purcell et al. 2012). In this survey, as much as 60% of the holothuriid species were encountered in shallow (1–2 m) seagrass beds. Some of the most abundant species (*B. marmorata, Holothuria atra* and *H. scabra*) were noted in these areas but substrate preferences were apparently different. Bohadschia marmorata and *H. fuscocinerea* tend to aggregate in seagrass areas with fine to silty substrate with patches of the algae *Halimeda opuntia*. In contrast, *Actinopya lecanora, H. atra, H. leucospilota* and *H. scabra* were encountered in sandy seagrass beds and coralline flats having coarse to fine substrates. Although regarded as nocturnal species, *B. marmorata* and *B. vitiensis* (Conand 1998; Purcell et al. 2012) were also encountered early in the morning with the sun out already. In Guam, *B. vitiensis* was also spotted during the day within the sandy lagoon at depths of 5–7 m (Michonneau et al. 2013). It is possible that these species tend to respond to temperature because during the reconnaissance survey done at around 20:00, no individuals were seen in the area where their aggregation was noted earlier. It could be attributed to the warmer temperature of the water at that time. However, further investigations must be conducted to verify this observation.

The same distribution with it conspecifics in Indo-Pacific were displayed by *Stichopus* species in coralline flats and reef areas (Conand 1998; Purcell et al. 2012). During the survey though, *S. noctivagus* was noted only once, due to the nocturnal and cryptic behaviour of this species. This species has not been reported in earlier studies conducted in the Philippines (Domantay 1934, 1962; Tan-Tiu 1981; Reyes-Leonardo 1984; Jeng 1998; Lane et al. 2000; Schoppe 2000a; Akamine 2005) until 2006 when Kerr et al. (2006) documented it in Central Philippines. *Stichopus rubermaculosus* is also a nocturnal and cryptic species observed foraging on sandy substrate with dead corals and rubble. Distinguished by the red spots on its dorsum papillae with brown-black patches (Massin et al. 2002), this species was only recently reported in Palawan, Philippines (Jontila et al. 2014). In contrast, *S. vastus* was quite common in coral reefs and areas having hard substrates. It is among the commercially processed sea cucumber in the country (Schoppe 2000b; Akamine 2005; Purcell et al. 2012).

For most sea cucumbers, distribution is associated with feeding and protection (Mercier et al. 2000; Dissanayake and Stefansson 2012). As such, detritus feeders and burrowing sea cucumbers such as

Actinopyga, Bohadschia and *Holothuria* species were abundant in seagrass beds of Stations 1 and 2. Similarly, cryptic species and those feeding on sediments and benthic algae such as *Holothuria coluber, P. graeffei* and *Stichopus species* were found on coralline flats and coral reefs where their food is abundant. For *H. atra* and *S. chloronotus,* it was found that in addition to bottom coverage, current strength is also a major factor in their distribution (Uthicke 1994). Having a suitable settlement site is also another factor in the recruitment of sea cucumbers (Eriksson et al. 2012). Studies have shown that seagrass beds are important settlement areas for sea cucumbers inhabiting shallow and intertidal areas (Friedman et al. 2012; Dissanayake and Stefansson 2012). This could possibly explain the high diversity and density of species noted at Stations 1 and 2.

Studies have also shown that as re-workers of sediments, sea cucumbers are important in maintaining the productivity of an aquatic ecosystem (Lampe 2013). In coral reefs, the ammonium excreted by benthic holothurians enhance the microalgal assemblage (Uthicke and Klumpp 1997), which also contributes to the overall production of coral reefs (Sorokin 1993). Burrowing dendrochirotids also increase the benthic primary production as organic nutrients become available for benthic microalgae (Wolkenhauer et al. 2010), and the survival of other species is affected by changes in their density (Birkeland 1988). This could be correlated to higher diversity of associated species observed in the seagrass area of Station 1, wherein at least eight seagrass species were identified: *Cymodocea rotundata, Enhalus acoroides, Halodule pinifolia, H. uninervis, Halophila minor, Syringodium isoetifolium* and *Thalassia hemprichii).* Macroalgae, particularly *Halimeda* and *Padina* spp., as well as invertebrates (*Cyprea* species, crabs, sea urchins, sand dollars) and even fishes (*Plotosus lineatus*) were also common at Station 1. In contrast, far fewer numbers of associated species were sighted at Station 2, sea cucumber distribution is sparse. Only four species of seagrasses were recorded, including *C. rotundata, E. acoroides, H. uninervis* and *T. hemprichii.* Other flora and fauna were also less abundant at this station. In coral reef areas, much higher numbers of species could probably be encountered if more stations and transects were established. The occurrence of rare species presents an opportunity for further studies of these species and others that have not yet been fully investigated.

So far, no article has been published on the population of sea cucumbers in Palawan in order to compare with the results of this study. But based on Jontila et al. (in this issue), it appears that Arreceffi Island has higher density estimates of sea cucumbers than coastal municipalities in Palawan that are open to exploitation. These estimates are much higher than those recorded in the Bolinao-Anda reef system in Pangasinan (Olavides et al. 2010) and Tubbataha Reefs Natural Park (TRNP) (Dolorosa and Jontila 2012). This could probably be due to the high exploitation rate in these areas, except in TRNP. The impact on sea cucumbers from overharvesting has been well-documented. For instance, in Wadi Quny and Eel Garden in the Red Sea, *Bohadscia marmorata* and *B. vitiensis* were exploited to depletion after a decade of fishing (Hasan and Abd El-Rady 2012). Similar trends were noted in areas with open access such as Lomaiviti, Fiji and in shallow lagoons of Mauritius where sea cucumber densities were only 0.83 and 8.14 ind. 100 m^{-2}, respectively (Lalavanua et al. 2014; Lampe-Ramdoo et al. 2014). For *B. marmorata* and *H. atra,* their high densities could partly be due to their ability to reproduce sexually and asexually through transverse fission (Conand 1995; Laxminarayana 2005, 2006; Purwati 2009). It is also possible that they are aggregating in this area since the survey was conducted in February, which is the peak of their spawning season (Purcell et al. 2012). Similarly, *H. edulis* and *H. leucospilota* were also abundant in sandy reef areas outside the transects. Reichenbach and Holloway (1995) noted that these species also reproduce by fission.

Conclusion

The recorded population of sea cucumbers at Arreceffi Island in Honda Bay, Palawan, Philippines indicates that the area is a critical habitat for these species. The occurrence of aggregations of *Bohadscia marmorata* and *Holothuria scabra* indicates successful spawning in the area. Although juveniles were barely noted due to the difficulty in finding them, the presence of suitable substrates and the high population of adults and sub-adults suggests that the island could be a source of larvae, which could help revive populations of sea cucumbers in nearby depleted sites around Honda Bay, and that recruitment could be high in the area. Overall, the results of this study highlight the importance of protecting the habitat in conserving sea cucumber species. Protection could go along with tourism, as long as it is properly managed. Creating a marine protected area is often prompted with implementation issues due to financial and management problems. Having an area for tourism that generates income for its protection appears to be a good option. The Arreceffi Island and TRNP prove that tourism, protection and conservation work together.

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Present status of the sea cucumber fishery in Turkey

Mehmet Aydın[1]

Introduction

More than 66 sea cucumber species are commercially fished worldwide (Purcell et al. 2012). The Mediterranean Sea is thought to have 37 species from the Holothuriidae family (Fischer et al. 1987). So far, eight holothurians (*Holothuria forskali, H. mammata, H. polii, H. sanctori, H. tubulosa, Stereoderma kirschbergii, Stichopus regalis* and *Synaptula reciprocans*) have been recorded in Turkish waters (Aydın and Erkan 2015; Aydın 2015).

The sea cucumber fishery in Turkey started in 1996 with the export of *S. regalis,* which was a bycatch species of shrimp trawls during the years 1996 and 1997. After 1997, three species (*H. tubulosa, H. mammata* and *H. polii)* became the main targets of the sea cucumber fishery (Aydın 2008; González-Wangüemert et al. 2014), but between 2002 and 2009, *H. tubulosa* and *H. mammata* became the two major commercial sea cucumber products of the Turkish seas. The sea cucumbers are not domestically consumed and are, therefore, used as an export product, mainly to Asian countries (Çaklı et al. 2004; Özer et al. 2004; Aydın 2008; Aydın et al. 2011). This article presents information about the present status of the sea cucumber fishery in Turkey. Data were obtained from the General Directorate of Fisheries and Aquaculture, SUBİS (Fisheries Information System) records and private companies.

Fishing areas and management

The sea cucumber fishery is only allowed in the northern Mediterranean region by Turkish regulations (Fig. 1). This region is divided into two subregions, one of which is left as a recovery zone (alternating every four years) to let stocks recover. The use of rotational zoning systems has been reported as a successful method in the management of sea cucumber stocks (Purcell et al. 2016). Prior to opening a fishing zone in Turkey, the status of the stocks (e.g. individual weights, lengths, biomass, distribution) is checked by the Turkish Ministry of Food, Agriculture and Livestock, and if it is appropriate, then fishing is allowed.

Moreover, fisheries in an open region are not allowed during the reproduction period (1 June –1 November). Vessels intending to catch sea cucumber must be authorised by the Ministry of Food, Agriculture and Livestock at the beginning of the fishing season.

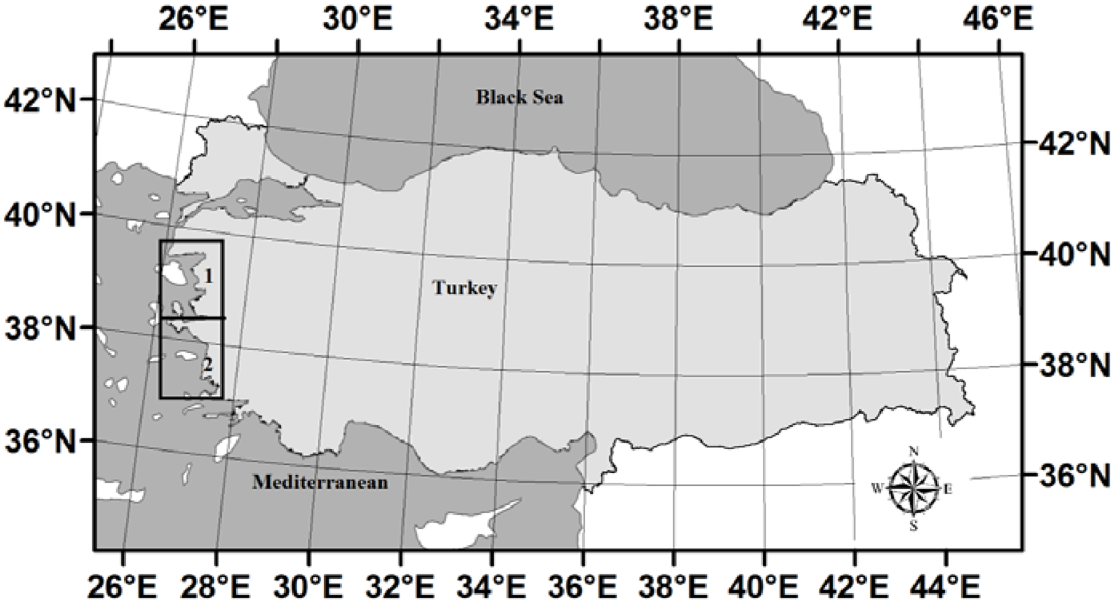

Figure 1. Sea cucumber fishing areas in Turkey shown by rectangles. Source: Fisheries Information System 2016

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Fishing method

Wooden boats, 4–12 m long and with 28–170 hp engines are typical in the sea cucumber fisheries of the region (Fig. 2). On average, two divers work on each vessel, diving to depths of 3–30 m using a hookah system (surface supplied air). In 2008, a single diver's catch was anywhere from 2,000 to 3,000 individuals per day (Aydın 2008), a figure that is comparable to today's catch amounts.

Table 1. Number of boats authorised to fish for sea cucumbers in Turkey.

	2008	2009	2010	2011	2012	2013	2014	2015	2016
Number of boats	46	69	45	87	89	29	46	50	109

Note: It is estimated that an additional 30% of illegal boats are also fishing for sea cucumbers in recent years.

An ordinary vessel using hookah is equipped with a compressor, air tank, air hose and regulator as well as bags and barrels used to catch and store sea cucumbers. Vessels with a hookah system use one or two divers simultaneously to collect sea cucumbers. In the latter case, air is divided by a hose with a "Y" shape piece leading to two separate air regulators.

When the vessels reach the fishing sites, they either anchor or drift. When anchored, the divers can operate in an area that is limited by the length their hoses. When they finish harvesting in a zone, the vessel moves to another place for another dive.

Figure 2. A sea cucumber fishing boat from İzmir.

When the weather permits, the vessel is left drifting and the divers are able to collect sea cucumbers from a wider area. The collected sea cucumbers are kept in bags by the divers. The bags are tied to upturned barrels, which are filled with air using the regulator to keep them neutrally buoyant in the water column as the harvest weight increases. This helps divers moving the barrels around under water. When a bag is filled with cucumbers, the barrel is fully filled with air so that it rises to the surface. Fishing with the hookah system is conducted in the daytime, and generally, a diver stays underwater for three to four hours, depending on the depth.

Processing

Sea cucumbers collected by the divers are transferred to the processing plants in plastic barrels (Figs. 3 and 4).

Figure 3. A sea cucumber catch brought aboard and bagged.

Figure 4. Plastic barrels used to transfer the harvested sea cucumbers to the processing plant.

Sea cucumbers are transferred live from the sea water to the processing plant, where they are first eviscerated by squeezing the body from the ends towards a 2 cm ventral incision (Fig. 5).

After evisceration, the sea cucumbers are washed with cold water and then boiled under pressure at 100°C for 20 minutes (Fig. 6).

After boiling, the material is washed again with cold water (Fig. 7).

The Asian demand for sea cucumbers is constantly increasing (Purcell 2010; Perez and Brown 2012; Roggatz et al. 2015). Depending on the buyer's request, sea cucumbers are usually marketed as frozen, cooked-dried, cooked-salted, cooked-salted-dried and cooked-calcium removed products (Figs. 8–12). In recent years, sea cucumbers harvested and processed in Turkey are mostly exported to Taiwan, Hong Kong and the United States in frozen or dried form.

Sea cucumber production in Turkey

Turkey's yearly sea cucumber production since 1996 is given in Table 2.

Total production during 1996–1997 was based on an estimate of commercial catches of *Stichopus regalis* as bycatch of the shrimp beam trawls. However, this species was no longer commercially harvested after 1997.

There was a gradual linear increase in sea cucumber production up to 2010, and in 2011 production

Figure 5. Evisceration of sea cucumber at the processing plant.

Figure 6. Pressurized boiling of sea cucumbers.

Figure 7. Boiled and cold-washed sea cucumbers.


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Figure 8. Sun-drying sea cucumbers.

Figure 9. Sea cucumbers in a drying room.

Figure 10. Frozen sea cucumbers.

Figure 11. Salted sea cucumbers.

Figure12. Calcium removal, a chemical method that is a company secret.


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Table 2. Total production of sea cucumbers in Turkey by year and product.

Year	Total production (kg)	Product	
1996	19,868	Frozen	
1997	37,665	Frozen	
2002	172	Pulverised (for the use in pharmaceutical industry)	
2003	10,843	Dried and pulverised	
2004	5,421	Dried	
2005	53,293	Dried and frozen	
2006	24,200	Frozen and dried	
2007	77,238	Frozen, dried and salted	
		Dried (kg)	Frozen (kg)
2008	33,669	789	32,880
2009	37,976	7,036	30,940
2010	97,183	16,203	80,980
2011	479,985	13,930	466,055
2012	447,644	27,479	420,165
2013	254,226	21,465	232,761
2014	247,585	23,585	224,000
2015	270,270	51,300	218,970
2016 (first six months)	150,250	100	150,150

Data comes from private companies and the General Directorate of Fisheries and Aquaculture.

soared. The reason was that *H. polii* had not been commercially exploited up to 2011 due to its low economic value. However, the processing of this species began that year, which increased total sea cucumber production. Moreover, fishing effort approximately doubled in 2011 and 2012, which is reflected in production amounts. The trend since then has been relatively stable.

Conclusions

Sea cucumber fisheries in Turkey have been managed by legal regulations since 2007. Sea cucumber fishing is allowed only in a small area of the northern Mediterranean where a fallow period goes into effect after four years of fishing. Resumption of fisheries after the fallow periods is allowed only after a stock assessment has been made by the Ministry of Food, Agriculture and Livestock. Boats engaged in sea cucumber fishing must obtain legal authorisation before every fishing season. These regulations seem to have allowed a sustainable sea cucumber fishery in the region.

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Processing techniques for white teatfish *Holothuria fuscogilva* and black teatfish *H. whitmaei* in Fiji[1]

Ravinesh Ram,[2] Roveena V. Chand[3] and Paul C. Southgate[4]

Introduction

The sea cucumber fishery is an important source of income for coastal communities in the Pacific (Conand 1989). Holothurians or sea cucumbers are consumed as a delicacy and for their perceived medicinal properties, and are particularly sought after in Southeast Asian markets (Bordbar et al. 2011; Esmat et al. 2013). The global trade in sea cucumbers is based on whole, gutted and dried sea cucumbers, commonly known as beche-de-mer (*iriko* in Japanese, *hai – som* in Chinese or *trepang* in Indonesian) (Bumrasarinpai 2006; Ferdouse 1999; McElroy 1990). From approximately 1,200 known species of sea cucumbers, around 58 are traded on Asian markets (Li 2004; McElroy 1990). The majority of commercially exploited species belong to the genera *Actinopyga*, *Bohadschia*, *Stichopus*, *Thelenota* and *Holothuria*, with Asian buyers particularly targeting species from the genus *Holothuria* (Li 2004). Sandfish (*Holothuria scabra)*, white teatfish (*H. fuscogilva)* and black teatfish (*H.* nobilis in the Indian Ocean or *H. whitmaei* in Asia Pacific) are among the highest value species (Holland 1994) in Asian markets where well-dried "A" grade product commands prices of around USD 70–190 per kg according to size and quality (McElroy 1990).

Beche-de-mer processing entails an uncomplicated sequence of actions resulting in a product that is non–perishable if stored in dry, dark conditions. The processing techniques currently used for beche-de-mer in Fiji were developed in the 1800s and have changed little since then. Post-harvest steps include first boiling, slitting and gutting, second boiling, smoking and finally sun drying (Holland 1990; Kinch 2002; Li 2004; McElroy 1990; Purcell 2014b; Sachithananthan et al. 1985; Seeto 1999; SPC 1994). Although these steps are uncomplicated, they require continuous attention to obtain a standardised dry product, and failure to do so can result in reduced quality and value of the final product (Purcell 2014b; Sachithananthan 1985; SPC 1994).

Processed white teatfish (*H. fuscogilva*) is ranked as the most valuable and superior product from Fiji's sea cucumber fishery, with a value of more than USD 140 per kg dry in Asian markets (Purcell et al. 2012). White teatfish replaced sandfish (*H. scabra*) as the dominant and most valuable product from the Fijian beche-de-mer industry in 1989, when Fiji's natural sandfish stocks collapsed because of overexploitation. Black teatfish (*H. whitmaei*) is currently the second most valuable product from Fiji's sea cucumber fishery in Asian markets. It is favoured for its relatively thick tegument (~12 mm).

A number of studies have reported the general processing methods used for sea cucumbers (Ram et al. 2014a) but there is limited literature reporting on specific processing methods used for individual sea cucumber species (Sachithananthan et al. 1985). Factors affecting the quality of beche-de-mer have been poorly studied. There is a particular lack of information relating to the influence of processing methods on the value of beche-de-mer in Asian markets (Battaglene and Bell 2004; Conand 1990 and 2004; Li 2004; Purcell 2014b).

Sea cucumber harvesting and trading are increasingly becoming restricted in the Pacific Islands region because of overexploitation. Even where stocks are still healthy enough to keep sea cucumber fisheries open, it is important to make sure that the value of the catch is maximised by proper processing techniques. This paper details for the first time the proper processing technique used by some processors in Fiji for white teatfish and black teatfish. If standardised, this method will help processors increase the value of their white and black teatfish products. They should then be able to give a better price for these sea cucumber species to fishermen, who will therefore need fewer sea cucumbers to make the same income. Hopefully, the end result will be less pressure on wild stocks.

Processing methods for teatfish

Information for this paper was collected online and through personal observations at the processing stations of local sea cucumber processors and beche-de-mer exporters in Fiji.

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Harvesting

White and black teatfishes are found as deep as 30–40 m (Reichenbach 1999) in Fiji and generally deeper than the majority of commercially collected sea cucumber species. Fishers collect the two species by free diving and by using scuba, despite the fact that it is an illegal sea cucumber fishing method in Fiji (Carleton et al. 2013; Pakoa et al. 2013). Divers hold harvested sea cucumbers in a porous cotton or polyester sack and, because of their relatively high value, both species are held separately from other species that are harvested from the same site (Ram et al. 2014c).

Once the divers return to the surface and to the vessel from which they operate, harvested sea cucumbers are kept alive in containers filled with seawater; they are maintained separately from other harvested seafood items such as crustaceans. This reduces potential damage to harvested sea cucumbers (i.e. skin scratches and cuts), which would affect the quality and value of processed sea cucumbers. Before processing, white teatfish and black teatfish are laid on a flat surface to obtain a cylindrical shape and to relax the muscles in the body wall (Fig. 1). This relaxation is important for the subsequent cooking step, because if sea cucumbers are put in hot water immediately after being harvested, the body wall can burst open and this would have a major impact on the product quality and value (Ram et al. 2014a). The relaxing process generally takes about 15 min.

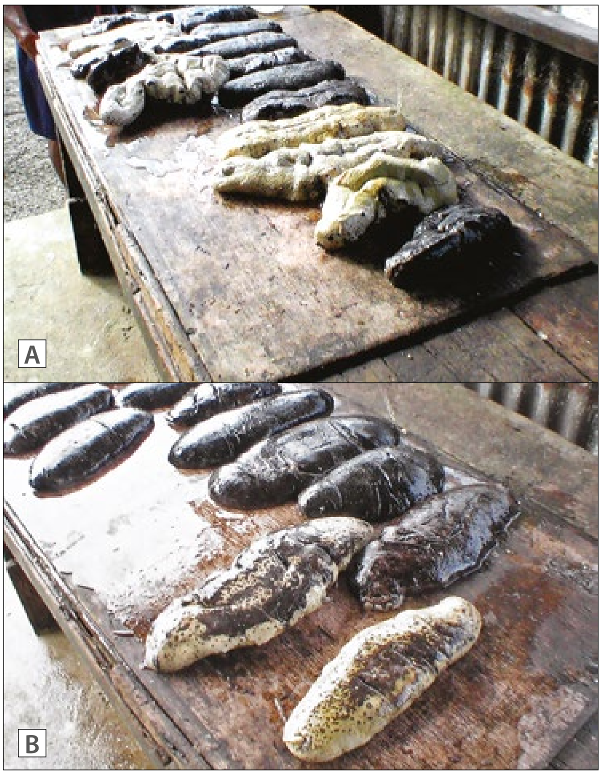

Figure 1. Freshly harvested white teatfish and black teatfish (A) laid on a flat surface to obtain a cylindrical shape and to relax the muscles in the body wall (B) before processing.

First cooking

Sea cucumbers are sorted before cooking to ensure that all sea cucumbers of the same size are cooked together. A large pot of fresh water is generally heated above an open fire. The temperature of the water must be 40–50°C before sea cucumbers are immersed individually (Fig. 2A). A wooden stirrer is used to prevent damage to the sea cucumbers during the cooking process and to assist with their even cooking.

The skin of both white teatfish and black teatfish are very fragile and wrinkles quickly if immersed in very hot water. Immersing sea cucumbers slowly and one by one also reduces the water temperature and the fire must be intensified for the water to maintain the suitable cooking temperature. The sea cucumbers are cooked at 40–50°C for 15–20 min. with occasional stirring. After this cooking period, the fire is intensified and the water temperature is increased to 80–90°C. Sea cucumbers are then cooked until they begin to swell, attaining a cylindrical shape and becoming harder and less rubbery. The whole "first cooking" process takes approximately 30 min. to complete.

Figure 2. Initial cooking of white teatfish and black teatfish (A) and products after the first cooking (B).

Figure 3. Slitting and cutting white teatfish and black teatfish on the dorsal surface. Note: the space left from the mouth to the slit on one side and from the anus to the slit on the other side.

Cutting and gutting

After completing the first cooking, sea cucumbers are removed from the cooking pot and arranged on a clean dry surface to cool for around 30 min. (Fig. 2B). Using a sharp knife, a neat cut is made on the dorsal surface of the cooked sea cucumbers, ending around 25 mm from the mouth and the anus at either end (Fig. 3). Once the cut is complete, the viscera are removed and the resulting cavity is cleaned. In Fiji, a faulty cutting step in sea cucumbers processing is a major contributing factor to quality and revenue losses (Ram et al. 2014b). For example, for both white and black teatfish, Fijian fishers often cut from the anterior to posterior end thus opening the entire gut cavity.

Salting

The salting step is used to speed up the drying process and to preserve sea cucumbers from spoiling. In Fiji, a bag of salt costs around FJD 30.00 (±USD 14.50), which is expensive for local fishers. As a result, sea cucumber fishers rarely use salt for the high value species they collect. After cleaning and gutting, salt (Grade 11, coarse solar salt) is pushed into the gut cavity (Fig. 4A) and into the two ends near the mouth and anus until there is no exposed surface that could be prone to spoilage. Teatfish are then turned upside down with the ventral surface facing upwards (Fig. 4B) and covered in salt. The sea cucumbers are spaced so that salt can be packed tightly between them before they are covered entirely with salt. Salting is done in containers that have tiny holes to allow moisture to drain from the sea cucumbers during the dehydration process. The salting container is held in an inclined position to facilitate drainage and is covered with a polyester cover to prevent external moisture entering the container. Teatfish are kept in salt for 48–60 h.

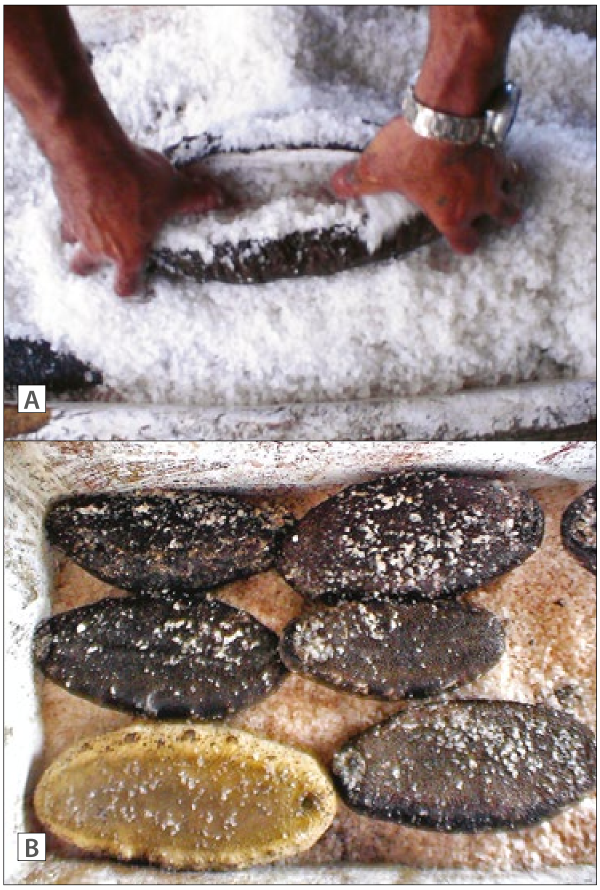

Figure 4. The salting procedure used for teatfish showing salt being pushed into the gut cavity (A) and individuals covered with salt (B).

Second cooking

After salting, teatfish are boiled a second time and the "second cooking" is of a longer duration than the "first cooking". Salted sea cucumbers are washed free of salt using running fresh water and then immersed one by one into a cooking pot containing hot water at 40–50°C.

They are left at this temperature for around 10 min. and then the water is brought to the boil for a further 10–15 min. Introducing salted teatfish to boiling water directly damages their skin and reduces the quality of the final product. The skin of teatfish is fragile after salting. After the boil, teatfish become hard and attain a cylindrical shape (Fig. 5), indicating that the second cooking is complete. The products are then prepared for sun drying.

Sun drying

To facilitate sun drying, a small stick of wood, around 50 mm long, is used to hold open the cavity of each sea cucumber (Fig. 6), which is then left in the sun with the dorsal slit surface facing upwards. The drying process continues for 5–6 days (with warm sunny days) or may take longer with less

Figure 5. White teatfish cooked twice with a perfect shape.

Figure 6. Sun drying white teatfish and black teatfish. Note: small lengths of wood are used to hold open the gut cavity to facilitate the drying process.

favourable weather. During the night, the products are stored in an enclosed room with fans. During the drying process, the teatfish become shorter and the tegument thickness shrinks from around 12 mm to 6–8 mm. As the product dries, a salt crust forms on the skin surface and this must be removed with fresh water before drying continues. If this salt crust is not removed, it attracts external humidity and compromises the drying process. After washing, the length of the pieces of wood holding open the gut cavity is reduced to around 25 mm so that the cavity can be closed properly after the final cooking.

After about 5–6 days of drying, the sea cucumbers become hard with a tough texture, indicating that the product is ready for the final cooking. Once the gut openers are removed, the teatfish are returned to the pot for the third and final cooking.

Third cooking

The third cooking is done primarily to assist with straightening the sea cucumbers and maintaining a cylindrical shape, which maximises their quality. During the third and final cooking, the gut cavity closes and the product attains a neat cylindrical shape (Fig. 7B). The final cooking last for only 5–10 min at a temperature of 60–96°C before a final sun drying with the gut cavity closed (Fig. 7B).

Final drying

Drying of the final product is further enhanced using an oven at night to prevent spoilage until the product has lost a considerable amount of moisture. Sea cucumbers in their driest state have only 8–10% moisture (SPC 1994). To monitor the drying process, beche-de-mer processors apply pressure to the outer skin of the product. Sea cucumbers that are not fully dried emit a "squeaky" noise and are left to continue drying. Properly dried sea cucumbers make no noise when pressure is applied and have a very hard outer surface. This indicates that the product is fully dried and can be packed after grading.

Figure 7. Final drying of teatfish in an oven (A) and in the sun until the final product is very hard (B).

Packaging and grading

Before packaging, the final products are graded according to size, shape, quality and appearance (Table 1). They are then stored in polyester sacks (Fig. 8) that are sealed and stored in a cool, dry place. The dried sea cucumbers are hygroscopic and absorb moisture if exposed to high levels of atmospheric humidity.

Figure 8. Packaged sea cucumbers.

During the processing of sea cucumbers accidents do occur, particularly when processing large batches. Common problems include over-cooking or over-drying, depending on the size of the sea cucumbers. These incidents have a significant effect on the final product quality and value. High value species, including teatfish, are graded into four categories or grades before packaging (Fig. 9).

"A" grade beche-de-mer generates the highest revenue while "D" grade product generates only a fraction of this revenue (Table 1). "C" and "D" grade beche-de-mer can also be produced by marine products agents, particularly if processing is done on a large scale, and white teatfish and black teatfish are cooked with no prior sorting of different sizes. This often results in larger animals being cooked properly while smaller animals are over-cooked and become distorted during the drying process. Where processing is done by the sea cucumber fishers themselves in remote areas where resources such as salt are unavailable, the resulting

Table 1. Grading system used for processed white teatfish (WT) and black teatfish (BT) in Fiji, and their approximate values.

Grade	Product description for grading	Price per kg of dried WT*	Price per kg of dried BT *
A	Very large size, straight in shape, neat appearance, neatly cut, white and brown spot coloration, teats intact, odorless and cylindrical	USD 141–274	USD 106–139
B	Medium size (≥7.62 cm or 3'), little distortion to shape, gut cavity open, may contain debris (sand and viscera), zigzag cut, teats damaged, white and brown spot coloration	USD 60–90	USD 45–53
C	Smaller size (<7.62 cm or 3'), shape distorted, debris present (sand and viscera), gut cavity open, skin wrinkled, cuts made from mouth to anus, teats damaged, faded colours	USD 40–60	USD 30–45
D	Very small size, shape distorted, presence of debris (sand and viscera), irregular cut, holes in tissue as a result of over cooking and burst, skin wrinkled, teats damaged, faded colours	USD 15–40	USD 15–30

* Prices from Purcell et al. (2012) and Pakoa et al. (2013).

Figure 9. Different grades of beche-de-mer processed from high value species of sea cucumbers. A): Grade A; B): Grade B; C): Grade C and D): Grade D.

product is generally reprocessed by marine product agents resulting in C–D grade products (Fig. 9) of low value (Table 1).

Conclusions

White teatfish and black teatfish are the two dominant high value species of sea cucumbers utilised for beche-de-mer production in Fiji. This study outlines in detail for the first time the processing procedure for these species in Fiji. This process takes up 3–4 weeks to obtain a high-grade product, and lack of attention during processing can have a significant negative impact on product quality and value (Ram et al. 2014a). Processing manuals are available to assist fishers and processors to maximise the quality and value of the beche-de-mer that they produce (Purcell 2014a). However, the information provided is often generic and does not fully cover species-specific requirements relating to the sequence, conditions (e.g. boiling temperature) and duration of processing steps that are required to produce the highest quality beche-de-mer from white teatfish and black teatfish. The results of this study fill this knowledge gap. It is hoped that the method described here will be widely adopted in Fiji to improve product quality and increase revenues generated by this important coastal livelihood activity and export industry.

Acknowledgements

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Sea cucumber fisheries in Northeast Brazil

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For decades, tropical sea cucumber fisheries have centered on the Indo-Pacific region (Shepherd et al. 2004; Tuwo 2004; Uthicke 2004) and have only recently developed in Latin America (Toral-Granda 2008). This expansion is mostly dictated by a sustained demand from Asian markets, especially China (Yang et al. 2015). Despite the fact that this is a fast-growing sector, relatively little is known about the sea cucumber industry in Central and South America. Only a few countries (e.g. Cuba, Peru, Chile, Mexico and Ecuador) have regulated fisheries, and provide production data (Toral-Granda 2008); for example, for *Isostichopus fuscus* in Baja California, Mexico (Aguila-Ibarra and Ramirez-Soberón 2002). To our knowledge, reviews of sea cucumbers in Latin America (e.g. Toral-Granda 2008; Anderson et al. 2011) do not provide any information about the state of sea cucumber resources and harvests in Brazil. This contribution aims to report on the occurrence of sea cucumber fisheries, and describe common practices to collect and process sea cucumbers in northeastern Brazil, thereby adding one new species of sea cucumber (*Holothuria grisea*) to the list of commercial species known around the globe (Purcell et al. 2013; Leite-Castro et al. in press).

Two types of survey were carried out. Ground surveys (8–17 September 2014) were conducted to confirm that the exploited species was, in fact, *Holothuria grisea* (Fig. 1), which is one of the most abundant species along the Brazilian coast, occurring from the northeast to the south of Santa Catarina (Tommasi 1969; Mendes et al. 2006). Ground surveys were restricted to the northeast of Brazil, covering an area of 2,611 km^2, which is equivalent to 35.3% of the Brazilian coast. Four states were found to harbour populations of sea cucumbers: Bahia, Paraiba, Rio Grande do Norte and Ceará. Among them, Ceará and Rio Grande do Norte (Fig. 2) had the greatest densities of *H. grisea*.

Following the ground surveys, aerial observations were made to identify fishing sites along the coast using a helicopter (EC 130 B4) that flew 100 m over the coastal areas that are believed to have the highest densities of sea cucumber drying areas in Ceará State. Four helicopter surveys were conducted around the full moon of 14–17 October 2014 (period during which the coastline is most exposed), covering 470 km of coastline, corresponding to 87% of the Ceará coast. The flights coincided with low tide and aimed to identify all types of sea cucumber fishing

Figure 1. The sea cucumber *Holothuria grisea* collected in the intertidal zone along the coast of the state of Ceará in northeastern Brazil. This individual is about 15 cm long. (Image: J. Souza Jr).

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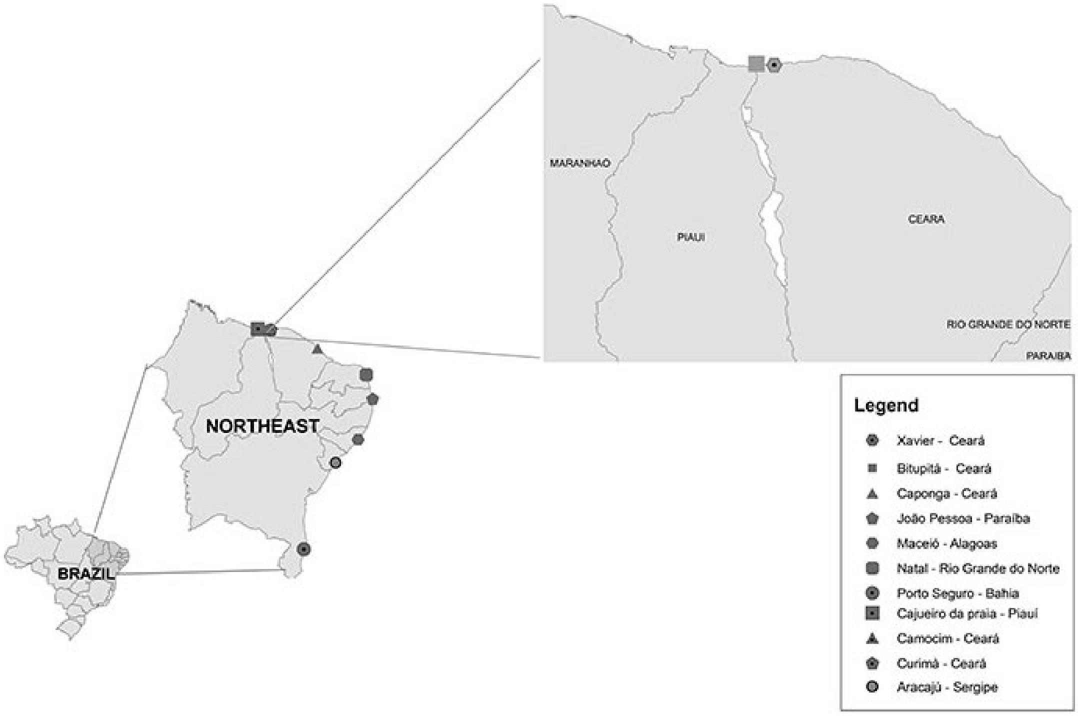

Figure 2. Maps showing the states of northeast Brazil where surveys were conducted and locations where sea cucumber fishing activities have been identified. (Illustration by M. Fernandes).

activities. A sea cucumber drying area was located on Xavier Beach, in Camocim, Ceará (Figs. 2, 3a). This community was then used to exemplify the sea cucumber fishery.

To characterise the types of fishing activities and the methods used by the local population to harvest sea cucumbers, Xavier Beach was monitored between 13 and 18 July 2015 during the lowest tides of the month (which correspond to the harvest period). The studied community is located in a remote area that is difficult to access and has only 26 homes.

The sea cucumber fishery is not a traditional activity in this region; historically, harvests have focused mainly on fish, shrimp and lobster. Sea cucumber collection in this community started 18 years ago, but intensified rapidly over the last 8 years, as a complementary income activity of traditional fisheries. Sea cucumber harvests take place twice a month, at low tide, when the reefs are exposed, and involve 14 fishing families, composed of men, women and children. The daily fishing starts before low tide, with the harvesters using a boat to reach the reefs (Fig. 3b), and ceases when the reefs are no longer exposed. Fishing spots are spread along the shore or near the coast, with distances from land ranging from 10 to 2,000 meters (from the highest high tide mark). Accessibility is facilitating the participation of novice harvesters (Fig. 3c).

Sea cucumbers are collected by hand, and all sea cucumbers are processed shortly thereafter (Fig. 4a). Evisceration is carried out on the beach at low tide by the harvesters themselves, using a small knife; the process takes about 8 seconds per specimen (Figs. 4a-c). Afterwards, all sea cucumbers are stored in large bags (of about 50 L), and every harvester weighs his or her catch in a nearby house belonging to the community representative. During the collection period, which lasts five days (around each appropriate lunar phase), about 506 kg of eviscerated sea cucumbers are collected. According to the data gathered, it is estimated that this community alone collects around 12,144 kg of sea cucumber annually. Many other villages like this one harvest *H. grisea* every month, especially in Ceará State.

Production data are noted by a community representative and then passed on to the middleman. Each harvester gets paid daily for the delivery of fresh eviscerated sea cucumbers. In the absence of competition, the price is determined by the middleman, and is currently set at about USD 0.51 kg^{-1} of wet weight. Sea cucumber fisheries in the entire village produce an estimated annual income of USD 6,193, which is unequally divided among harvesters, depending on their level of experience and expertise. *H. grisea* is cryptic, living hidden inside crevices, but experienced fishers quickly detect the types of rocks that are used as shelter by the sea

Figure 3. A: Aerial view of sea cucumber drying area on Xavier Beach (Ceará, Brazil); B: Sea cucumber fishers readying their boat; C: Fishers wading on the reef in search of sea cucumbers. (Images B and C: J. Souza Jr)

Figure 4. A to C: Sea cucumbers are eviscerated on the shore immediately after the harvests; D: A boulder that was turned over, exposed sea cucumbers hidden underneath. (Images: J.-F. Hamel)

cucumbers. Unfortunately, the rocks that get turned over to collect sea cucumbers are not returned to their original position (Fig. 4d). This practice exposes hundreds of encrusting marine invertebrates, including sponges, tunicates and worms, many of which presumably die. Hence, this fishery is not only impacting on the sea cucumber population and their closest associates (e.g. predators), but also a myriad of other species that make up this rocky coastal community.

The processing, carried out by one of the fishers, involves boiling the eviscerated sea cucumbers in 100 L cast-iron metal cookware with about 2 kg of salt for two hours (Fig. 5a). The boiled products are laid on a perforated plastic box (36 cm x 55 cm x 31 cm) for approximately 12 hours to remove excess water. The sea cucumbers are subsequently scattered on canvas and left to dry under the sun for six consecutive days (Fig. 5b). Sometimes, in order to speed up the drying process (or during rainy days) a

Figure 5. A: Boiling in salted water is the first step in the preparation of beche-de-mer; B: After boiling, sea cucumbers are typically sun dried; C: On rainy days, sea cucumbers are dried using a wood-burning oven; D: The lengthy shore-based evisceration process and poor training often yield low-quality products that may be over-cooked, brittle, or misshapen. (Images: J. Souza Jr)

home-made oven is used (Fig. 5c). Steel plates set over a wood fire are used as shelves to accommodate approximately 560 kg of wet sea cucumbers. After the drying is completed, the resulting beche-de-mer is stored in Styrofoam boxes and transferred to the middleman. During the present survey, several examples of poor-quality dried products were noted, which were either contaminated by sand and dust, or not properly cut or burned (Fig. 5d). Based on interviews with fishermen, sea cucumbers are the main source of trade and income for their community, whereas other fish are used mostly for subsistence. Sea cucumbers are not consumed in Brazil, and the route used by exporters to ship them out of the country remains unknown.

The status of *H. grisea* populations was not characterised before the onset of the fishery along the coast of Brazil. Therefore, it is not possible to infer about the impact of this activity. However, studies of population densities conducted by the PEP-MAR group in 2009, on a beach close to the village of Bitupitá (Fig. 2), showed natural concentrations of 40 ind. m^{-2} (unpublished data). Another study performed by Farias (2012) in the same locality reported a density of 0.54 ind. m^{-2}, corresponding to a decrease of 98.7%. Such a drastic decline can result from a number of factors, including intensive fishing pressure. However, in-depth and controlled studies should be conducted in order to determine the precise effect of fishing activities on population densities. While biological investigations of *H. grisea* populations in Brazil have begun (Leite-Castro et al. in press), more research is necessary to understand the patterns of seasonal fluctuations in population density, as well as to characterise the fishery and monitor its productivity. Based on this information, management strategies could be developed to monitor fishing activities for *H. grisea*, which is clearly a major source of income for dozens of small villages scattered along the coast of Brazil.

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From life-sustaining to life-threatening: The case of the sea cucumber fishery in Nicaragua

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Over the last several decades, researchers have been increasingly concerned about overfished marine resources around the world (Jackson et al. 2001). Declines in natural stocks of several species have fuelled reflections on poor fisheries management and the unsustainable exploitation of seafood, at the expense of grander designs such as the well-being of future generations and the conservation of ocean biodiversity. Sea cucumber fisheries have been at the forefront of crusades for ecosystem and community-based exploitation. Harvests of tropical sea cucumbers have surged around the Indo-Pacific region in countries such as Indonesia, Papua New Guinea, India, Solomon Islands, Kiribati, and a constellation of tiny island states in Melanesia, Micronesia and Polynesia. The general consensus is that sea cucumber fisheries typically follow the alarming boom-and-bust cycle (Purcell et al. 2013, 2014). As the demand from Asian markets, specifically China, grows incessantly, so does the pressure exerted on resources. Concurrently, sea cucumbers constitute the fastest growing aquaculture sector, having generated close to 4 billion US dollars in revenue in China alone in 2015 (Yang et al. 2015).

In recent years, meetings organised by the United Nations (Food and Agriculture Organization) and the International Union for Conservation of Nature were tasked with exploring solutions to the sad fate of sea cucumbers worldwide, and orchestrating better transfer of knowledge and technology among stakeholders to protect the ever-more fragile sea cucumber resources. Horror stories consisted mainly of sea cucumbers locally going extinct and altercations between landowners, harvesters and government officials over quotas and licences. However, a very different spectre has recently started to creep over the sea cucumber industry. One that reeks of human injustice, abuse and even death, as it unfurls in the postcard perfect lagoons of Central America.

The present contribution reports on recent discussions with the Miskito Prince Jose Miguel Hendy Coleman in fall 2016. Aside from being an environmental activist, Mr Coleman is one of the many Miskito fishers who expressed concerns about the sea cucumber fishery in Nicaragua. As a prince, descendant of monarchic rulers of the former Miskito Kingdom, Mr Coleman is deeply affected and preoccupied by the illegal harvest of marine resources and, most importantly, by the avoidable loss of human life.

Sea cucumber fishing was introduced in Nicaragua by Honduran fishers. During the early 1990s, the number of vessels involved grew from 1 to 20 as the fishery expanded. In recent years, the primary species harvested include *Holothuria mexicana* and *Isostichopus badionotus*, which combined, constitute 90% of the catch. However, other products such as conch, lobster, shrimp and high-value fish species such as snapper, snook and kingfish are also harvested. Unsurprisingly, immature sea cucumbers now represent a growing and alarming proportion of the catches that get trucked to processing plants to be processed. They are primarily exported to the United States by Chinese exporters living in Nicaragua or exported to Honduras illegally or legally through the island of Roatan (off the cost of Honduras). Among the fishers' concerns are illegal fishing, taking undersized individuals, inhumane treatment, and the lack of other livelihood alternatives.

About twenty 15–18 m-long boats are known to fish daily along the cost of Nicaragua, primarily around the Miskito Cays. These islands lie ~72 km away from the fishing communities. Fishing trips may last up to 12 days, with activities carried out during the daylight hours (05:00–17:00). Each boat carries about 20–30 fishers and 10 crewmembers, including workers, cooks and the boat captain.

The fishery operation financiers (Chinese and Koreans) operate from nearby Honduras. Their main contacts in Nicaragua are the Nicaraguan boat owners who hire a boat captain, a scale operator (to weigh the catch inside the boat) and a "*sacabuzo*" (who hires the fishers); all of which are Spanish or Mestizo descendants (the latter being of mixed Spanish and native Indian descent). The *sacabuzo* hires the fishers who are all Miskito. Miskitos are

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descendants of the Indian natives, with African and British ancestry. Miskitos are also hired to work at the sea cucumber processing plants.

There are alarming reports of unethical and inhumane conditions aboard the fishing vessels, and the desperate poverty that drives these fishers to work in dangerous conditions. According to Mr Coleman, a worker can dive up to 17 times a day to depths of 70 m (with no decompression protocol), with a mask and carrying a tank in their arms, no regulator or weight, just trying to swallow the bubbles that are coming out of the tank! Therefore, each dive lasts about 10–15 minutes per tank. At times, two fishers dive using a shared tank. The age of fishers ranges from 15 to 45 years; about 20% of them are 15–19 year-olds who are "trained" on two to three dives by the older fishers. After this "training", they are unofficially certified to fish. The fishing grounds consist primarily of 7,000 ha around the Miskito Islands, which are the native fishing grounds of artisanal Miskito fishers.

According to Mr Coleman, these unregulated fishing methods result in at least one to three injuries or fatalities daily. Fishers express concerns over the fact that injured or dead co-workers are not returned to shore until two to four days after the fact or, sometimes, until the last fishing day. During this time, the bodies of the Miskito fishermen who died from decompression accidents are reportedly preserved on ice along with the catch. The fishers suffering from decompression sickness (dizziness, hallucination) that can make it back to the harbour are finally taken to a hyperbaric chamber. However, Mr Coleman indicated that the boat captain typically does not report the true fishing depth (often reporting that fishing occurred at 30 m only). Therefore, the chamber can be set at the wrong pressure, which can result in a fisher's death.

Those lucky enough to survive their injuries often have to go through further ordeals. According to Mr Coleman, when a fisher seeks compensation for decompression disability from the boat captain, the latter refers him to the sacabuzo who hired him. The *sacabuzo* then refers the fisher to the boat owner who might refer him to the owner who lives in Honduras. Unable to contact the true operation owners, the disabled fishers often have no other resort but to retire without receiving any compensation.

Another main concern reportedly expressed by fishers is that boat owners do not allow inspectors, government officials or non-governmental organisations to inspect or search their boats. Sea cucumbers barely fetch USD 6.00 kg^{-1}. Wives and mothers are allowed to collect their husbands' and sons' pay in advance but interest is charged. Alleged items carried in the fishing boats include drugs such as marijuana, which fishers can exchange with their catch; meaning they can obtain these items instead of getting paid for their work. Fishers claim that these drugs provide them with the courage to dive deeper to find more sea cucumbers.

The fishers are also concerned about the inhumane treatment that prevails during fishing trips. Mr Coleman reported that boat captains have been known to throw meals on the deck for workers to pick up and eat. Food provided includes beans, rice, chicken, beef, pork and primarily fish which is at times not very well cooked. Moreover, the boats themselves are generally dirty and unsecure. There are even stories of fishers complaining of exhaustion who got punished; the boat captain would place tissue paper between their toes and light it to warn others not to complain. When asked why they continue to fish despite the risks and mistreatments, fishers apparently indicate that it is the only job available to them in Nicaragua. To draw attention to the problem, Mr Coleman conducted a video interview on 30 November 2016 with Miskito fisher Gary Venegas Valerio from Bilwi Nicaragua who was injured while harvesting sea cucumbers.[4]

The negative impact of this exploitive practice is manifold. On one side, the sea cucumber resources are severely depleted and the artisanal Miskitos' fishing grounds are degraded, consequently affecting their livelihood and income. Fishers are faced with having to sell their meagre catches of lobster, conch and fish to the same boat owners that oversee the sea cucumber fishery. They are further financially affected from being paid based on how much they catch, especially when the person operating the scale underestimates the harvests. Worst of all, the families of invalid or deceased sea cucumber fishermen are left struggling with overwhelming psychological and financial problems.

Offering the tiniest sliver of hope, Nicaraguan fishermen have expressed interest in sea cucumber aquaculture. They have also expressed a desire to learn about the biology and ecology of the sea cucumber species they currently fish and to get more information on methods of harvesting sea cucumbers. Perhaps they just need a bit of help from the government of Nicaragua to overturn a bleak and untenable situation. We have decided to make a contribution through this short article, in

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[4] The interview can be seen on YouTube at: https://www.youtube.com/watch?v=mT-BizpDIqA

the hopes of helping the Miskito from Nicaragua get a fair chance at a brighter future.

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We would like to thank Jose Miguel Hendy Coleman for sharing this life story with us. We dedicate this text to all the people in Nicaragua and elsewhere who fish for sea cucumbers, sometimes under very precarious conditions.

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Large-scale sandfish (*Holothuria scabra*) aquaculture in multitrophic polyculture ponds in southern China

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Abstract

The sea cucumber "sandfish" (*Holothuria scabra*) is now produced in southern China within multitrophic polyculture ponds (>300 ha) with pearl oysters and groupers. Starting stocking densities are 2–3 ind. m^{-2}, and final densities at harvest are in the range of 95–320 g m^{-2} of sandfish in the ponds. Current production is over 1 million market sized animals annually. China is outpacing Indo-Pacific island countries in the commercial application of mariculture technology for culturing sandfish.

Introduction

Mariculture of the high-value sea cucumber sandfish, *Holothuria scabra* Jaeger 1833, has been possible for more than a decade owing to the development of hatchery and grow-out technology (e.g. Pitt 2001; Agudo 2006). Commercial-scale aquaculture is occurring in countries such as Madgascar, Vietnam, Maldives and Saudi Arabia (Purcell et al. 2012), and has recently taken off in southern China. Apart from relatively small-scale production in New Caledonia, and recent community-based ranching in Papua New Guinea (Hair et al. 2016), there has been very little uptake of hatchery and grow-out technology in other Pacific Island countries.

In China, sandfish is called *ming yu shen* (明玉参), or "rough sea cucumber" (*cao hai shen*, 糙海), and is naturally occurring in China's tropical waters of southern Guangdong Province. Since around 2010, sandfish have been cultured in Xuwen County, near Zhanjiang City, on the western coast of Guangdong Province (approx. 20°15′N, 110°13′E).

Commercial production

Sandfish are grown-out in earthen seawater ponds (Fig. 1) on three farms, comprising more than 300 ha in surface area (5,000 mǔ = 307 ha).[3] Many of the ponds were previously used for shrimp culture, but were abandoned due to problems with heat stress in the ponds.

The large ponds in Xuwen County are being used to grow sea cucumbers in a three-dimensional polyculture with Akoya pearl oysters for mabé pearls (*Pinctada martensii* [Dunker]) and groupers (*Epinephelus* sp.). In 2015, approximately 4 million juveniles were produced for grow-out. The starting stocking density for grow-out is about 2–3 ind. m^{-2}. From these juveniles, 1–2 million commercial sized animals were reportedly harvested, with body lengths averaging about 20 cm and body weights ranging 300 to 500 g per piece fresh (Fig. 2). These values of production equate to

Figure 1. An earthen pond used to culture sandfish, pearl oysters and groupers. Pearl oysters are suspended on lines from plastic bottles floating on the surface.

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[3] A mǔ is a Chinese unit of area equivalent to 614.4 m^2

Figure 2. Fresh sandfish harvested from polyculture ponds in southern China.

final grow-out densities (at harvest) in the range of 95–320 g m^{-2} of sandfish in the ponds, which is commensurate with grow-out densities of sandfish in earthen ponds from other regions in the world (Purcell et al. 2012).

Conclusions

The mariculture proponent believes that the three-dimensional culture of pearls, sea cucumbers and fish in the ponds allow for better recycling of aquaculture feeds and waste products. Production volumes have increased dramatically in recent years through the testing of suitable stocking densities and pond maintenance strategies for the polyculture. Although much of the early development of hatchery technology for sandfish was developed by WorldFish in Solomon Islands and New Caledonia, other countries such as China are taking a lead with the large-scale commercial application of that technology.

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Market value of flower teatfish ("pentard"): A highly exploited Indian Ocean holothuroid

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Abstract

The large sea cucumber *Holothuria* sp. (type "pentard") is exploited extensively throughout the western Indian Ocean, yet little information exists on its market value at the distal end of the value chain. We collected data on prices and sizes of this species from 15 lots in 14 shops in Hong Kong and Guangzhou, China. This species was relatively common in the marketplace. Market values ranged from USD 44–273 kg^{-1} dried. Weight-to-length ratios were lower for pentard than white teatfish (*H. fuscogilva*), but only marginally so. Prices per piece increased dramatically as a function of product length, but the relationship was weak for price per kg. This market study verifies that pentard is highly valuable in Asian markets, and therefore is likely to be at high risk of overexploitation. Our results show that fishery income from harvests of pentard could be considerably higher in the long run by regulating catches to large-sized animals, using minimum legal size limits.

Introduction

Flower teatfish (*Holothuria* sp., type "pentard"), locally known as *pauni chui* in Zanzibar (FAO 2013) and *preema bathik attaya* in Sri Lanka (Dissanayake et al. 2010), is a large holothuroid apparently in the teatfish complex of species, in the subgenus *Microthele*. This putative species is still under taxonomic investigation (Muthiga and Conand 2014), and further studies are required to determine if it is a separate species or simply a variant of another teatfish species (*H. nobilis* or *H. fuscogilva*) (Conand 2008). Herewith called "pentard", as it is known in Seychelles, this animal is dark brown on the dorsal surface with mottled and irregular-shaped cream-coloured blotches with prominent teats on the lateral margins of the body (Conand 2008; Purcell et al. 2012). In contrast, the Indian Ocean black teatfish, *H. nobilis,* is black on the dorsal surface with white blotches and spots on the lower sides of the animal and around the teats, and white teatfish *H. fuscogilva* has a varied colour pattern, but is mostly whitish or beige with brown or grey blotches (Purcell et al. 2012). Pentard commonly inhabits sandy-bottom lagoon habitats at depths of 10–50 m (Conand 2008).

Pentard is reported to have a sparse distribution in the western Indian Ocean (Conand 2008), being reported from Seychelles, Comores Islands, Tanzania and Sri Lanka (Aumeeruddy and Conand 2007; Conand 2008; Dissanayake et al. 2010; Eriksson et al. 2010; FAO 2013; Muthiga and Conand 2014). This species is a sought-after target species in western Indian Ocean fisheries due to its high market price (Conand 2008; Eriksson et al. 2010; FAO 2013; Muthiga and Conand 2014), and is one of the main harvested species in Seychelles (Aumeeruddy and Conand 2007, 2008). The price offered to fishers can be similar to that of *Holothuria nobilis* and *H. fuscogilva* (Eriksson et al. 2010). In Seychelles, export prices in 2008 were USD 17–26 kg^{-1} (Aumeeruddy and Conand 2007). In some fisheries, pentard is collected by scuba diving, which could be a driving factor causing the depletion of this resource, along with its high value, poor knowledge about this species, and inadequate monitoring and enforcement of fishery regulations (Conand and Muthiga 2007).

In its dried form, pentard can be distinguished from white teatfish (*Holothuria fuscogilva*) by the numerous white spots and small blotches on a grey background (Fig. 1) (Aumeeruddy and Conand 2007). In contrast, dried white teatfish are mostly whitish in colour or whitish with some dark tan-grey spots. Dried pentard also have a more flattened body shape (see Fig. 1) when compared with white teatfish.

This study is the first published record of the value of pentard in key market hubs in China for internationally traded beche-de-mer. We aimed to determine whether market price varied as a function of the size of the products. The findings are instructive for fishers, traders and fishery managers.

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Figure 1. Dried pentard on sale in a) Hong Kong, and b) Guangzhou.

Methods

Data on market prices of pentard were collected at 14 stores in the dry seafood market in Sheung Wan district, Hong Kong and Yide Lu markets, Guangzhou, China similar to data collection methods by Purcell (2014). Access to the products in stores and language interpretation were facilitated by a Chinese member of the Australian Centre for International Agricultural Research. At the Hong Kong market, the unit weight was *catty* (604.79 g), whereas unit weight of products in Guangzhou was *jin* (500 g).[5] Prices were later converted from HKD and CNY to USD using international exchange rates at the date of sale. Stores in Guangzhou sold mainly in wholesale volumes, whereas stores in Hong Kong sold in retail volumes.

Within stores, we selected one or two lots of dried sea cucumbers, which were in tubs, jars or large bags (Fig. 1). From each lot, four randomly sampled specimens were measured: specimen length to ±0.5 cm along the ventral surface with a ruler, and weight to ±1 g using an electronic balance.

The average product (body) lengths and weights of the four subsamples from each lot were used as the data for analyses. Linear regression was used to examine the relationship between average product length and price per unit weight or price per specimen. A two-tailed t-test was used to test for differences in the weight:length ratio between pentard and white teatfish (*H. fuscogilva*).

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[5] *Catty* and *jin* are units of measurement in China.

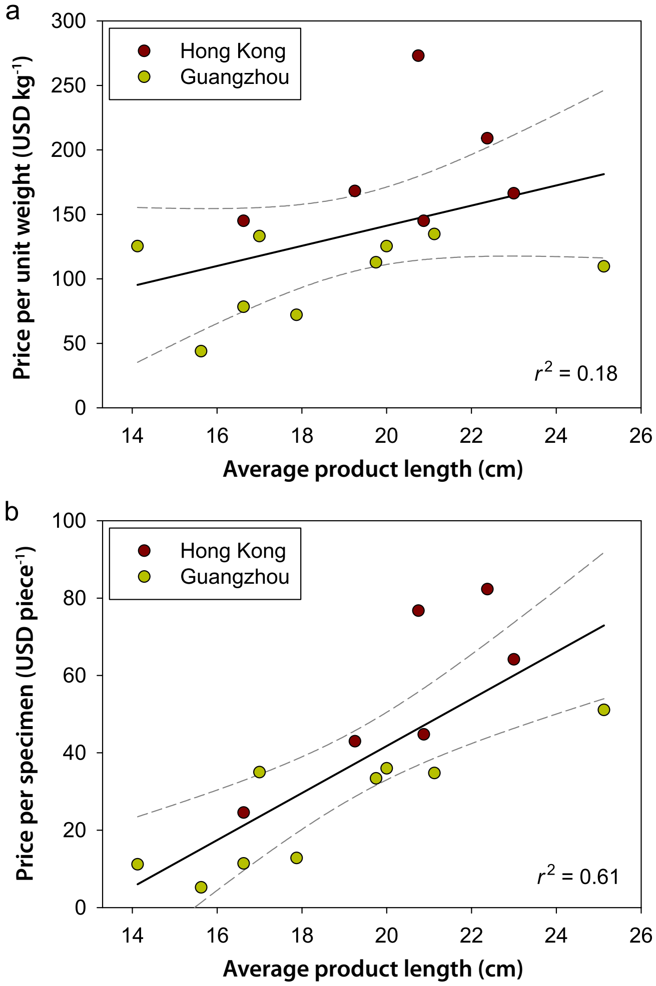

Figure 2. Scatterplots of a) average product length vs price per kilogram, and b) average product length vs price per piece. Data points are average product lengths from four subsampled specimens from lots within stores, and the corresponding prices of those lots. For b), price per piece is calculated from average product weight of the subsamples. Solid lines are the linear regressions; dashed lines represent the 95% confidence interval.

Results and discussion

Average lengths of dried pentard ranged from 14.1–25.1 cm among lots sampled, corresponding to average product weights of 89–466 g. The weight(g):length(cm) ratio for pentard was 12.9 ±0.5 s.e. ($n = 60$). By comparison, teatfish were heavier for their length, with the weight(g):length(cm) ratio averaging 14.1±0.3 ($n = 289$), but the ratios were marginally non-significantly different between the two species ($t = 1.74$, $p = 0.082$).

The market price for pentard ranged from USD 44–273 kg^{-1}. The average price for lots that we sampled was USD 136 kg^{-1}. As found previously for other species, prices tended to be higher in Hong Kong, which is more of a retail market (Purcell 2014).

Prices per unit weight increased somewhat with size of the specimens (Fig. 2a), but this trend was weak and statistically non-significant ($p = 0.12$). In contrast, the relationship between product length and price per specimen was stronger ($p < 0.001$), with length accounting for 61% of the variation in price per piece among samples (Fig. 2b). The relationship predicts that dried products of 14–16 cm will yield market prices of USD 5–17 per piece, whereas dried products of 22–24 cm will yield market prices of USD 54–66 per piece. The higher price range is due to the multiplicative effect of product weight.

Considering that dried pentard are 60–63% of the length of corresponding live animals (Aumeeruddy and Conand 2007), our study suggests that fresh animals of 23–26 cm will sell (in Chinese markets) for just USD 5–17 per piece when dried, whereas fresh animals of 36–39 cm can eventually command market prices of USD 54–66 per piece. This finding gives a strong reason for imposing minimum size limits for pentard in Indian Ocean fisheries, e.g. 30 cm fresh, 20 cm dried. The income from the fishery is likely to be significantly greater by protecting the animals from fishing until they are of a large size (assuming low natural mortality). Pentard was one of the exploited species unable to be assessed by the International Union for Conservation of Nature because it was "known only by common names and not yet described taxonomically" (Conand et al. 2014). In addition to resolving the taxonomic identity of pentard, resource management needs research on rates of growth and natural mortality to further winform decisions about appropriate minimum size limits.

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Reproductive biological characteristics and fatty acid profile of *Holothuria mammata* (Grube, 1840)

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Abstract

In southern Europe, the interest in sea cucumbers is relatively recent, but several fisheries have now been developed, as well as aquaculture projects. *Holothuria mammata* is one of the holothurian species targeted. In this study, some reproductive biological characteristics of *H. mammata* were studied, such as sex ratio, weight, gonadosomatic index, gonadal tubules morphology, and oocytes diameter in different stages of maturation. The lipid profile was also analysed in order to highlight the fatty acid requirements in a diet for broodstock conditioning. The spring period, from March to April, corresponds to maturation and subsequent spawning. Total fat content was 1.00% ± 2.45, and highest values of fatty acids were obtained for stearic acid (C18:0) (8.91% ± 1.44), arachidonic acid (C20:4 ω6) (19.97% ± 1.30), and eicosapentaenoic acid (C20:5 ω3) (10.85% ± 0.37). The findings lead us to consider higher requirements of long-chain polyunsaturated fatty acids (or LC–PUFAs) in broodstock diets, and their potential uses as functional foods and nutraceuticals. It is crucial to develop studies to increase the biological knowledge of *Holothuria mammata*, and create conditions to domesticate broodstock of this species and improve the rearing of sea cucumbers in Europe.

Introduction

Interest in sea cucumber species from the Mediterranean Sea is fairly recent, and focues particularly on *Holothuria arguinensis, H. mammata, H. polii, H. sanctori, H. tubulosa* and *Stichopus regalis* (Aydin 2008; Ramón et al. 2010; Navarro et al. 2012). These species are currently being fished in Turkey, Spain and France and traded in Asian markets (Chakly et al. 2004; Vannuccini 2004; Aydin 2008).

Sea cucumbers are also consumed for their beneficial effects on human health. Several studies in the last two decades have demonstrated that sea cucumber extracts possess biological attributes that promote wound healing and exhibit antimicrobial, antioxidant, and anticancer properties (Beauregard et al. 2001; Jawahar et al. 2002; Roginsky et al. 2004; Ogushi et al. 2015; Tian et al. 2005; Hing et al. 2007; Zhong et al. 2007; Li et al. 2008 a, b; Lu and Wang 2009; Janakiram et al. 2010; Bordbar et al. 2011; Santos et al. 2015 a,b). Furthermore, sea cucumbers are healthy food. They are rich in protein and low in fat, and are particularly rich in the essential fatty acids EPA (eicosapentaenoic acid), DHA (docosahexanoic acid), and ARA (arachidonic acid) (Fredalina et al. 1999; Chen 2004; Santos et al. 2015 a, b).

The present study aimed to characterise the reproductive biology of *Holothuria mammata*,by monthly examination of the gonadosomatic index (GI) and histological analyses of the gonadal tubules in order to improve the knowledge of this species for its aquaculture production. Total fat content and the fatty acid profiles were analysed.

Materials and methods

Sampling and gonadosomatic index

In total, 73 *Holothuria mammata* individuals were collected from the Peniche coast in Portugal (39°21′14.5″N and 9°23′43.4″W). The sampling procedure was carried out at low tide and sea cucumber individuals were transported alive to the Aquaculture Laboratory of MARE-IPleiria in Portugal. A longitudinal incision was made along the dorsal surface and the coelomic fluid and gonads were removed. Drained body weight (dwt) and gonad weight (gwt) were measured, and gonads were fixed in 10% buffered formalin for 24 hours. The gonadosomatic index (GI) was calculated using the following equation (Ramofafia et al. 2000):

$$GI = \frac{gwt}{dwt} \text{ x } 100$$

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Macroscopic examination of the gonadal tubules, and histological analyses

A maturity scale, based on macroscopic examination of the gonadal tubules, was followed and included: stage I – indeterminate, stage II – growing, stage III – mature, stage IV – partly spawned and stage V – spent (Ramofafia et al. 2000).

Ten tubules of each gonad were removed, and their length and diameter measured. For histology, the gonadal tubules were dehydrated and embedded in paraffin. Sections (6 μm thick) were stained with haemotoxilin and eosin, and five gametogenic stages were defined, following previous works, as recovery, growing, mature, partly spawned and spent (Ramofafia et al. 2000, 2003; Shiell and Uthicke 2006; Navarro et al. 2012). The diameter of oocytes was measured with LAS Leica imaging software on a Leica DM microscope (Leica, Bensheim, Germany).

Quantification of total fat content and fatty acid profile

Lipid extraction and fatty acid profile

The total lipid extraction method was adapted from Bligh and Dyer (1959) following a dry weight basis. Fatty acid methyl esters were prepared according to the methods of Lepage and Roy (1986) and Masood et al. (2005): 0.015 mg of crude fat was dissolved in 5 mL acetyl chloride:methanol (1:19 v/v) and heated in a water bath at 80 ºC for 1 h. Then, 1 mL ultrapure water and 2 mL n–heptane were added and the solution was vortex-stirred for 1 min followed by centrifugation at 1,500 g for 5 min. The organic upper phase was recovered and analysed by gas chromatography (GC).

A Finnigan Ultra Trace gas chromatograph equipped with a Thermo TR–FAME capillary column, an auto sampler AS 3000 from Thermo Electron Corp. (Boston, Mass., U.S.A.), and a flame ionization detector were used to quantify fatty acid methyl esters. Fatty acid methyl esters were identified in comparison to an external standard, fatty acid methyl ester mix (PUFA No 3 from Menhaden oil) was purchased from Supelco (Bellefonte, PA, USA).

Statistical analysis

A chi-square test ($\chi2$) was done to evaluate the differences in relation to the unit. Also, two-way analysis of variance ANOVA, followed by a *Bonferroni* multiple comparation test, was performed on the GI and weight (gutted).

For all statistical tests, the significance level was set at $p \leq 0.05$. All statistical tests were performed with IBM SPSS Statistics 23.

Results

Sex ratio and weight

The sex ratio obtained, 3:4, did not differ significantly from the unity ($\chi2(1) = 0.961$; $p > 0.05$). The gutted body weights of individuals varied from 60–140 g for males, 85–135 g for females, and 50–140 g for individuals lacking gonads.

Gonadosomatic index

The monthly gonad maturation pattern showed a high peak in April for both sexes. From January until April there was an increase in the GI values for both sexes, followed by a decrease in May and a steady increase in June. It was also noted that the GI values did not vary greatly between individuals, suggesting that the pattern of spawning may be synchronous among sexes. The maximum GI values obtained for April were 13.87% for females and 14.11% for males. No significant differences were detected between the GI values of both sexes ($F(1, 45) = 0.009$; $p > 0.05$) and months ($F(2, 45) = 2.238$; $p > 0.05$), nor was there a significant correlation between sexes and months ($F(2, 45) = 0.363$; $p > 0.05$).

The gutted body weight did not fluctuate significantly over the months except for the individuals lacking gonads. No significant differences were detected among the months ($F(2, 45) = 0.138$; $p > 0.05$) and in the interaction between sexes and months ($F(2, 45) = 2.655$; $p > 0.05$).

Gonad morphology

The gonads of both sexes consisted of a saddle-shaped gonad base from which extended numerous branching tubules of varying sizes. The gonoduct opened externally at the gonopore, on the dorsal side above the mouth. In total, five tubule size classes were defined based on tubule size and appearance (Table 1). Seasonal changes were observed. Overall, gonad growth involved the formation of new tubules arising from the gonad base, with a subsequent increase in tubule length and diameter. This was never, however, observed at stage I (indeterminate). At stage II (growing), both females and males could be identified by the presence of developing oocytes and spermatocytes. As the gonads approached maturity, tubule colouration also changed, depending on the stage of maturity. In mature females the tubules appeared orange. Mature gonads were bright orange in colour and tubules had transparent thin walls through which oocytes were visible. In mature males the gonads were whitish. Tubule length was a good indicator of the reproductive maturity stage, with females generally having a longer tubule length than males.

Table 1. Five maturity stages of *Holothuria mammata*, based on gonad tubule morphology (n=51).

Maturity stage and sex	Gonad wt (g)	Tubule				
		Length (mm)	Diameter (mm)	Branching	Condition	Colour
I						
indeterminate	Not identified					
II						
Females	6.4–12.8	29.8–43.6	1.8–2.1	1–3	Growing oocytes (32.3–50.6 µm)	Orange
Males	5.8–15.1	34–40.4	0.3–1.7	1–3	Developing sperm	White
III						
Females	7.8–22.1	36.4–50.6	2.1–2.2	1–4	Oocyte visible through tubule wall (108–122 µm).	Bright orange
Males	8.9–25.6	35.2–41.7	2.3–3.5	1–4	Tubules packed with sperm	Creamy white
IV						
Females	4.8–7	38.6–43.9	1.7–2.1	1–4	Reduced tubules, relict oocytes present, empty lumen visible	Bright orange
Males	3.2–6.9	29–41.6	2.1–2.3	1–4	Unspawned tubules with residual spermatozoa	White
V						
Females	2.1–5.3	16.2–18	2.3–2.5	1–4	Tubules shrunken and wrinkled in size. Relict oocytes.	Orange (transparent)
Males	3.3–5.4	16.4–22	2.4–3.3	1–4	Relict sperm presented	Transparent

Throughout the spawning season, the simultaneous presence of both spawned and unspawned tubules indicated that partial spawning was occurring (stage IV), which could be a characteristic of this species. Spent gonad tubules (stage V) were wrinkled and greatly reduced in size.

Females showed translucent and light pinkish colours during the growing phase (stage II), while orange or even reddish dominated during maturity (stage III).

Histology

The histological analysis revealed that the four gonad maturity stages (indeterminate stage was never observed) correlated well with the four stages of gametogenic development. A description of the histological features of each gametogenic stage is detailed below.

Females

Stage II: *growing*

The growing stage (Fig. 1A) was characterised by active vitellogenesis. Early and mid-vitellogenic oocytes were present. These oocytes had a distinct germinal vesicle. Vitellogenic oocytes were surrounded by follicle cells throughout development.

Stage III: *mature*

Mature ovaries were densely packed (Fig. 1B). The oocytes remained within their follicle and the germinal vesicle. An increase in oocyte diameter occurs and it is possible to visualize a well–defined nucleus.

Stage IV: *partly spawned*

It was observed that not all ovarian tubules released gametes during spawning. Partly spawned ovaries contained both spawned and unspawned tubules (Fig. 1C). Partly spawned tubules had a reduced diameter and a wrinkled appearance.

Stage V: *spent*

Spent ovaries were wrinkled and shrunken, with the presence of relict oocytes in the lumen (Fig. 1D), the gonad wall was thick.

Males

Stage II: *growing*

A striking feature of growing testis was the presence of numerous infolds of the germinal epithelium, which extent into the lumen (Fig. 2A). These infolds were lined by a dense layer of spermatocytes organised in short columns. It was observed in late growing-stage testis due to the growing number of spermatozoa abundance in the lumen.

Stage III: *mature*

The infolds of the germinal epithelium were reduced or absent and the lumen was packed with spermatozoa (Fig. 2B). A few spermatocytes were present along the germinal epithelium. The gonad wall was at its minimal thickness.

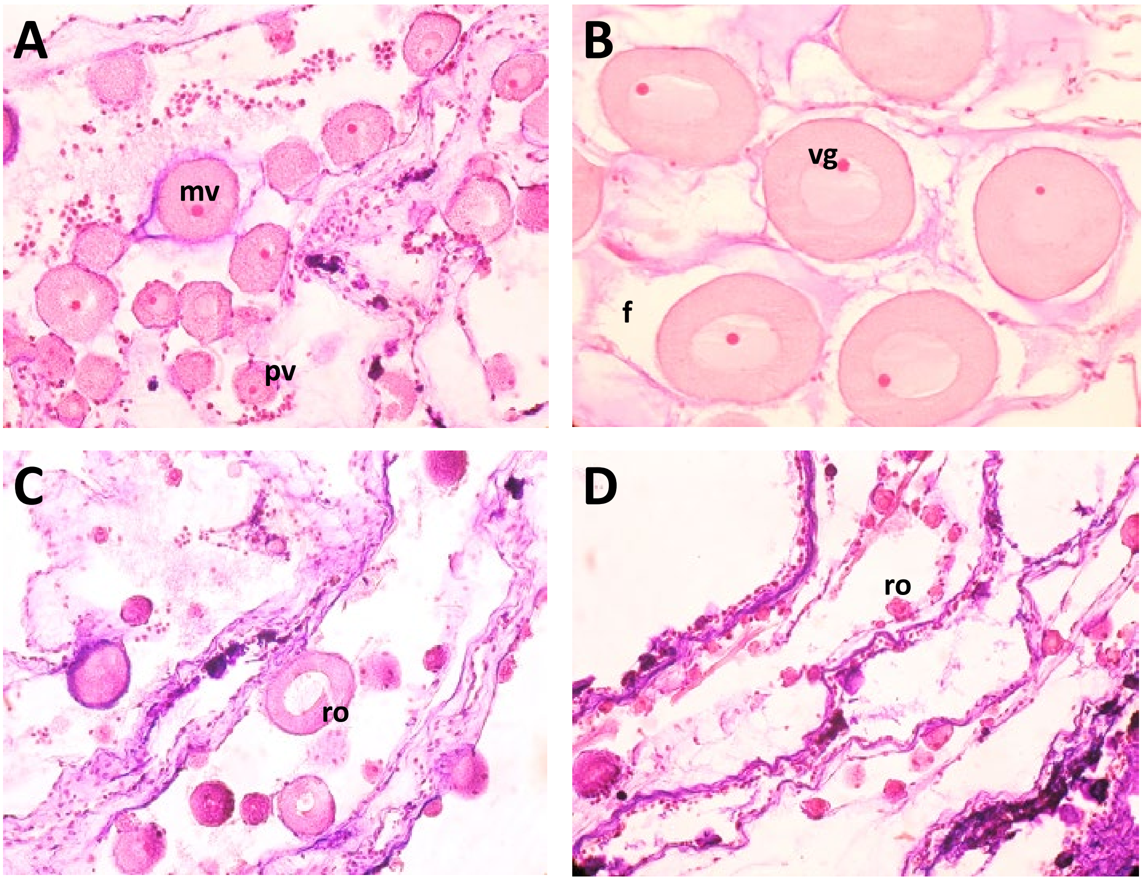

Figure 1. Oogenesis. **A**: growing ovaries, with oocytes in pre–vitellogenic (pv) and mid–vitellogenic (mv) stage. **B**: mature ovaries with oocytes enclosed within their follicle (f) and germinal vesicle (gv). **C**: partly spawned ovaries, with the presence of mature unspawned oocytes, relict oocytes (ro). **D**: spent ovaries with intensive shrinkage of the tubules occurred and a few relict oocytes persisted (ro). (Scale bars = 100 μm).

Stage IV: *partly spawned*

It was observed that not all gametes were released during spawning, and dense aggregations of spermatozoa and phagocytes were present in the lumen (Fig. 2C).

Stage V: *spent*

Spent tubules were shrunken and generally had an empty lumen, except for a few relict spermatozoa (Fig. 2D).

Quantification of total fat content and fatty acid profile

The total fat content for *H. mammata* was 1.00 ± 2.45%. Table 2 shows the fatty acid profile, with a high abundance of araquidonic acid (C20:4 ω6) (19.97 ± 1.30%), eicosapentaenoic acid (C20:5 ω3) (10.85 ± 0.37%), and stearic acid (C18:0) (8.91 ± 1.44%). The ω3:ω6 ratio obtained was 0.48.

Table 2. Fatty acid profile of *Holothuria mammata* by mean of fatty acid ± standard deviation (n = 3).

Fatty acid	*Holothuria mammata*
∑ SFA[a]	14.18 ± 1.68
C 12:0	0.65 ± 0.03
C 14:0	0.88 ± 0.02
C 16:0	3.74 ± 0.18
C 18:0	8.91 ± 1.44
∑ MUFA[b]	4.48 ± 0.26
C 18:1 ω7	2.89 ± 0.15
C 18:1 ω9	1.59 ± 0.11
∑ PUFA[c]	46.30 ± 2.18
C 16:3	7.27 ± 0.13
C 18:3 ω3	1.07 ± 0.19
C 20:2 ω6	6.35 ± 0.17
C 20:4 ω6	19.97 ± 1.30
C 20:5 ω3	10.85 ± 0.37
C 22:6 ω3	0.79 ± 0.02
ω3/ω6	0.48

a saturated, b monounsaturated, c polyunsaturated

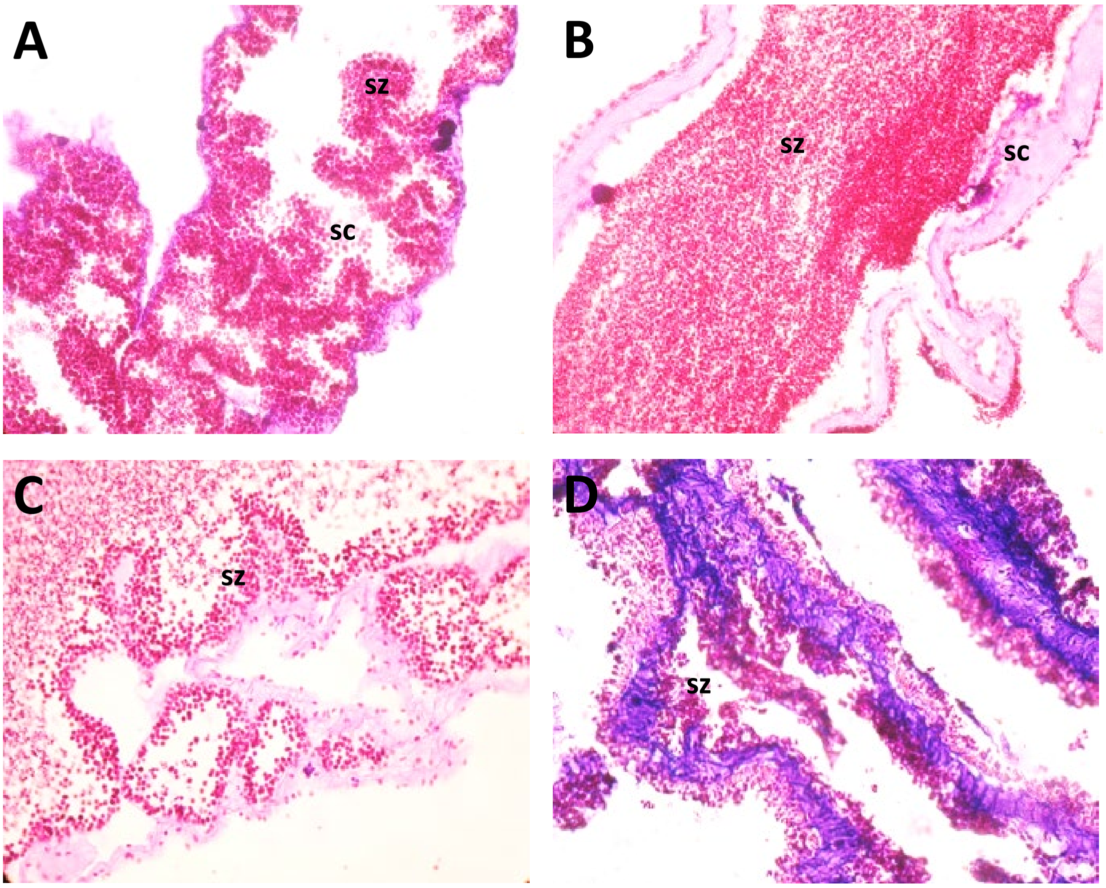

Figure 2. Spermatogenesis. **A**: growing testis with developing spermatocytes (sc) and spermatozoa (sz) beginning to fill lumen as part of the growth progress. **B**: mature testis with spermatocytes (sc) persisting along gonad wall and full spermatozoa (sz) accumulation. **C**: partly spawned testis, with spermatozoa (sz) in the lumen. **D**: spent testis with residual spermatozoa (sz) or empty lumen. (Scale bars = 100 μm).

Discussion

Although *Holothuria mammata* has a wide geographic distribution, data concerning its reproductive biology and biotechnological potential are not reported in the literature. There is only one study from Aydin et al. (2011) concerning the food potential of *H. mammata*. Therefore, our data were compared with those previously reported for similar species of the genus *Holothuria* or other species from the order Aspidochirotida of different geographic regions.

Sex ratio

The sex ratio obtained for *H. mammata* did not differ significantly from 1:1, although it has presented an unbalanced ratio of 3:4. In most holothurians from the order Aspidochirotida, the sex ratio often coincides with a balanced 1:1 ratio (Ramofafia et al. 2001; Rasolafonirina et al. 2005; Asha and Muthiah 2008). However, some species demonstrated an unbalanced ratio of 1:2 or 2:3, due to fishing pressure, which affects the population of a given area, or due to species that undergo asexual reproduction (Harriott 1982; Uthicke and Klumpp 1998; Uthicke and Karez 1999; Shiell and Uthicke 2006; Muthiga et al. 2009). In this study, the site where samples were collected is not considered to be a locality with high fishing activity. However, this site is seasonally affected by strong waves, which could be considered a factor affecting the balanced sex ratio.

GI and gutted body weight

Sea cucumbers often show one annual reproductive cycle (Cameron and Fankboner 1986; Smiley et al. 1991; Tuwo and Conand 1992; Conand 1993a,b; Chao et al. 1995), although semi–annual cycles (Harriott 1985; Conand 1993b) or even continuous reproduction activity throughout the year (Harriott 1985) are also frequent, particularly in tropical species. On the Peniche coast, *H. mammata* followed one single annual cycle, as would any typical temperate sea cucumber species (Sewell and Bergquist 1990; Tuwo and Conand 1992; Sewell et al. 1997). The maximum reproductive activity was observed in spring. For echinoderms, the annual reproductive cycle is clearly related to sea watr temperature as gametogenesis only occurs when sea water temperature rises. Generally, cold waters are associated with high nutrient availability that triggers phytoplankton blooms and favours larval development

(Boidron–Metairon 1995). Indeed, a synchronisation of echinoderms spawning according to phytoplankton availability is considered to be an advantageous adaptation (Starr et al. 1990), and our study revealed that the peak of gonad maturation was synchronised with phytoplankton availability, which in southern Europe is at a maximum in spring. The only observation of spawning individuals belonging to *H. mammata* was recorded at Gran Canaria (Spain) in August during a study by Navarro et al. (2012) for *H. sanctori*. This could suggest that *H. mammata*, like *H. sanctori*, reproduces in the months leading up to and including the summer. These observations may indicate that the reproductive cycles of *H. sanctori* and *H. mammata* may be synchronised, as reported for other echinoderms (Ramofafia et al. 2003; Shiell and Uthicke 2006; Muthiga et al. 2009).

The gutted body weights of both sexes showed slight differences between males and females. In general, females were heavier than males due to higher fecundity rates, requiring greater nutrient storage. The weight of individuals lacking gonads was generally slightly lighter than sexed individuals. This fact demonstrates the importance of gonads in establishing the weight of an individual, which is in agreement with observations made of other species (Morgan 2000; Ramofafia et al. 2000, 2003; Asha and Muthiah 2008; Navarro et al. 2012).

Macroscopic examination of gonadal tubules and histological analysis

The monthly assessment of gonadal tubule length showed some discrepancies between the sexes. It is a common feature of sea cucumbers for females to have a greater number of tubules (given its highest reproductive output) than males (Shiell and Uthicke 2006; Toral-Granda and Martinez 2007). However, it is described for a large number of species of the order Aspidochirotida, such as *Holothuria fuscogilva*, *H. nobilis*, *H. scabra*, and *H. atra*, that male gonadal tubules are slightly longer in length than female gonadal tubules (Conand 1993 b). Nevertheless, *H. mammata* counteracted this trend by having a distinct gonadal morphology that may be characteristic of the species or may possibly be related to the collection of young males with less weight and smaller gonads.

With regard to oocyte diameter, the range of values in stage III of oogenesis (mature) was 108–122 μm. These values are lower than the common values in tropical species, which range from 150 to 210 μm (Conand 1993b). However, the results are consistent with those observed by Tuwo and Conand (1992) for *Holothuria forskali*, where in stage III, the values were 90–120 μm. In the partly spawned stage, residual mature oocytes were present, and in stage V, trace oocytes were observed. These findings could indicate that spawning is not complete and different stages of oocyte development could be found throughout the year, as observed for other tropical sea cucumbers species (Ramofafia et al. 2000; 2003; Navarro et al. 2012).

Quantification of total fat content and fatty acid profile

A recent study by Aydin et al. (2011) on sea cucumber species in Turkey, including *H. mammata*, showed a substantially lower total fat percentage (0.09% ± 0.08) than the values obtained in our study (1% ± 2.45). This may be explained by seasonal changes in feeding behaviours and geographical variations (Chang-Lee et al. 1989) and, according to Ozer et al. (2004), the handling procedures are also likely to affect the chemical composition of sea cucumbers. Chang-Lee et al. (1989) have defined a range in total fat percentage for holothurians of 0.1–0.9%, but Santos et al. (2015a, b) report that the total fat content for *H. forskali* was 4.83% ± 2.33% and 3.63% ± 0.11% for *Stichopus regalis*.

Regarding fatty acid profile, in our study, *H. mammata* presented lower total percentages of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, than was reported by Aydin et al. (2011). On the other hand, *H. mammata* presented higher values of EPA, DHA and ARA fatty acids as well as of stearic acid (C 18:0). Several studies have shown that high values of the ω3:ω6 ratio have resulted in increased protection against degenerative and cardiovascular diseases (Russo 2009; Smith et al. 2009). According to FAO (2004), ω3/ω6 ratio should range between 1:8 and 2:5; the ratio observed for *H. mammata* in this study (± 1:2) is therefore consistent with those reported for other species.

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Gametogenesis, spawning and larval development of *Isostichopus* sp. aff *badionotus*

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Abstract

The sea cucumber family Stichopodidae is distributed throughout the Caribbean Sea. *Isostichopus* species are widespread in the entire Caribbean region, but little is known about the biology of this native species, especially on the Colombian coast. This study presents a description of the gametogenesis, natural spawning, and embryonic and larval development of *Isostichopus* sp. aff *badionotus*. Observed gonadosomatic index values were highest between August and November. Recovery, growth, mature, partially spawned and postspawning were the stages recorded by female gonad histology while in males, the observed stages were recovery, growth, mature and postspawning. The growth stages occurred in almost all of the sampling periods. During two consecutive years, natural spawnings were observed in the laboratory in the second half of each year (from July to November). Larval rearing reached the doliolaria stage. The animals were fed with a mix of *Thalassiosira* sp. (commercial food) and microalgae (*Nannochloropsis* sp). Larval mortalities were associated with infestation by copepods in the culture.

Introduction

Sea cucumbers are benthic marine invertebrates that are known worldwide for their ecological importance and economic value. During recent years, Caribbean sea cucumbers became the target in the search of sea cucumber species of a similar quality to those of Asian origin. But, sea cucumbers of the Colombian Caribbean Sea have not been well studied (Caycedo 1978; Borrero-Pérez et al. 2003; Toral-Granda 2008; Toral-Granda et al. 2008; Rodríguez et al. 2013; Agudelo and Rodríguez 2015; Vergara and Rodríguez 2016). There are many gaps in basic knowledge, including the reproductive biology of native Caribbean sea cucumbers. This study describes the gonadal morphology and gonadal development of wild *Isostichopus* sp. aff *badionotus*, including the spawning period, sex ratio, and evaluation of synchronisation stages of maturity between females and males. It also describes the larval development until the doliolaria stage. *Isostichopus* sp aff *badionotus* is roughly similar to I. *badionotus* but differs from it on some morphological and molecular (DNA) levels that will be discussed in another paper. The work provides records on I. sp aff *badionotus* reproduction, early development, and economic value, and constitutes a significant contribution to the biological knowledge of this of native sea cucumber species of the Colombian Caribbean. Our goal was to have a benchmark for *I.* sp aff *badionotus* reproductive characteristics in an aquaculture environment, for its sustainable management in the wild.

Material and methods

Collection of animals

Between February 2013 and January 2014 adult broodstock of Caribbean sea cucumbers were purchased from local artisanal fishers in Rodadero Bay (11°13′ 22,73″N and 74°13′32, 59″ W) and Airport Bay (11°07′10″N and 74°13′50″W) in Santa Marta, Magdalena, Colombia (Fig. 1). Sea cucumbers were quickly taken alive in 20-L plastic tanks filled with seawater to the Aquaculture Laboratory of the Universidad del Magdalena. On arrival, they were conditioned in 550-L tanks (temperature: 26 ± 0.6°C; salinity: 36 ± 0.8 ppt; oxygen: 5.36 ± 0.74 mg L^{-1}), where they were kept until the time of dissection. The next day, individuals were sacrificed by hypothermia and photographed. At that time, they were weighed (± 0.1 g) (using a Navigator-OHAUS scale, USA), and total weight (TW), drained weight (DW) and gonad weight (GW) were recorded.

Gametogenesis study

The gonadosomatic index (GI) was approximated in accordance with Abdel-Razek et al. (2005):

$$GI = \frac{gw}{dw} \times 100$$

In total, 120 individuals were examined microscopically during the sampling period. In the months during which the gonad weight was low (≤ 1 g), a complete analysis of gonadal material was carried

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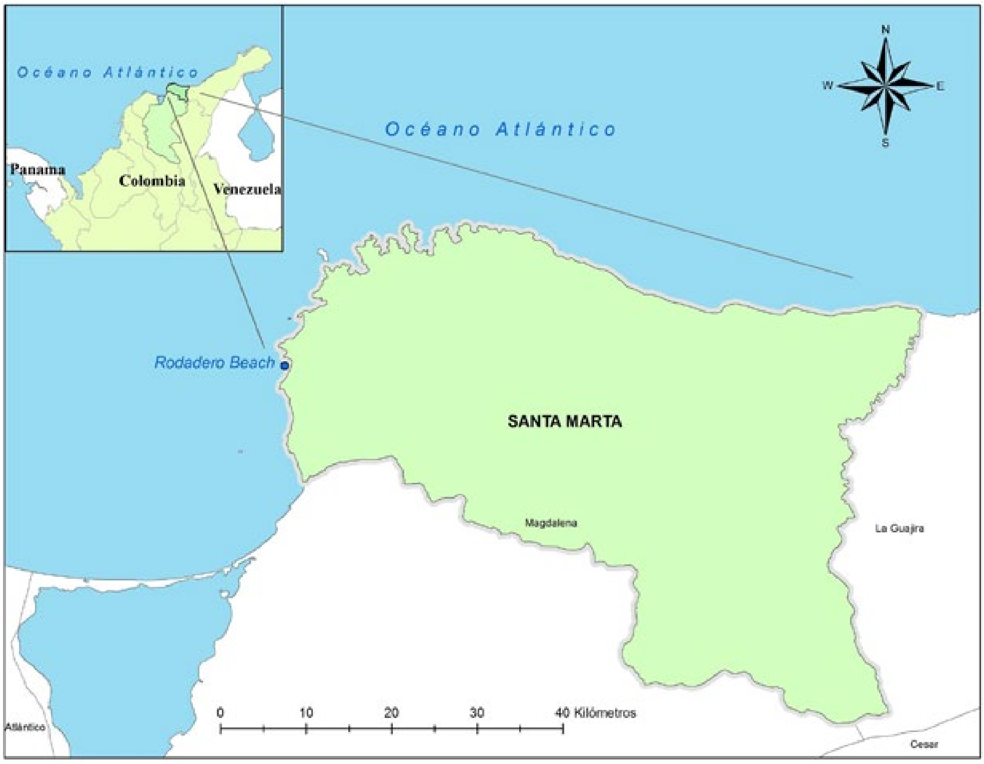

Figure 1. Marine and coastal areas of the Colombian Caribbean Sea (by Jose Viillacob). Blue point indicates the study area.

out, but in the months during which gonad weight was higher (> 1 g), one specific portion was selected for histological analysis (Fig. 2). The gonads were fixed in 10% formaldehyde in 0.1 M phosphate buffer (pH 7.2), for at least 24 h, then dehydrated in graded ethanol solutions and embedded in paraffin; they were subsequently sectioned in 5 microns lengths with a microtome (Sakura ® Accu-cut SRM), and the sections were stained with hematoxylin-eosin (H-E) (Drury and Walington 1980). A Zeiss microscope, equipped with camera AxioVision 4.8.2 software, was used to evaluate gametogenesis development and to measure the diameter of gonadal tubules and oocytes.

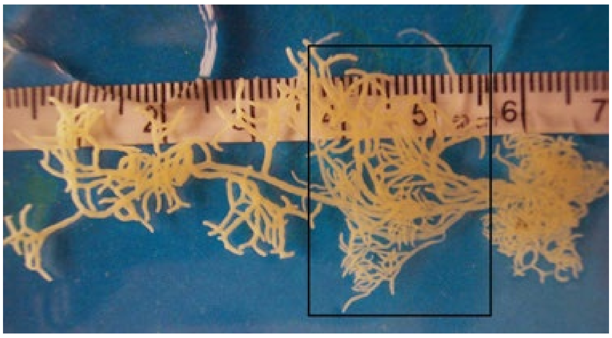

Figure 2. A fragment of gonadal tissue of *Isostichopus* sp. aff *badionotus* used for histological processing.

Sex determination, maturity stage and occurrence of reproductive events were assigned using the classification made by Ramofafia et al. (2000) and Fajardo-León et al. (2008).

Spawning and larval development

During the second half of 2014 and 2015, 100 adult sea cucumbers each year (232.09 ± 73.25g) were placed in ten 550-L tanks filled with sterilised seawater (temperature 26 ± 2.3°C; pH 7.8 ± 0.04; O2: 5.7 ± 0.22 mg L-1; salinity 36.5 ± 0.41 ppt), equipped with a biological filter, and aerated by air stones. The sea cucumbers were exposed to a 12-h light–dark photoperiod using overhead fluorescent lights. Faeces were siphoned out every day and water salinity was adjusted when needed. For broodstock maintenance, adults were fed with a mixture of marine sediment (previously washed and dried) and Spirulina powder (Artemia-International®) at a rate of 0.5 g per 100 g of sediment. Broodstock reproductive behaviour was monitored daily during the new moon phase. Two days before spawning, food was removed to avoid poor sea water quality (Agudelo and Rodríguez 2015). Natural spawning took place during the night or early in the morning. Successful fertilisation was recognised by the appearance of a fertilisation membrane. Fertilised eggs were siphoned and washed with filtered and sterilised sea water. To estimate the total number of eggs, samples were withdrawn using a 1-mL aliquot. After that, they were incu¬bated in 250-L tanks (1 larva mL^{-1}) at room temperature (26°C). Descriptions of the early development of *Isostichopus* sp. aff *badionotus* were made with the aid of light microscopy observations (Carl Zeiss, Modelo Primo Star), and photographic records were made with a digital camera (Axiocam ERC 5S). During 2013, the larvae were supplied with live algae (*Chlorella* sp.) at a concentration of 5,000 cells mL^{-1} but larvae did not grow (Agudelo and Rodriguez 2015). In 2014 and 2015, larvae were fed with a mix of live microalgae *Nannochloropsis* sp. and TW 1200 (Instant Algae® Products): early auricularia = 20,000 cells mL^{-1}; mid auricularia =40,000 cells mL^{-1} and late auricularia = 50,000 cells mL^{-1}. During 2015, we recorded the larval development until the doliolaria stage and described some abnormal eggs obtained.

Data analysis

Data from the GI were analysed by a simple analysis of variance test using Statgraphics program, where months were a factor and the variable response was the index value obtained in each of these months. To determine significant differences in the index during the months, nonparametric analysis of variance of Kruskal-Wallis (H) was applied, after verifying that the data did not meet the assumptions of normality (Kolmogorov-Smirnov test, $p < 0.05$) and homogeneity variance.

Results

Gametogenesis

Sea cucumbers had an average weight with standard deviation of 271.25 ± 84.93 g; while their average DW was 233.67 ± 78.10 g, and no significant differences were found between weights by gender: females had an average weight of 236.91 ± 82.40 g, while males had an average weight of 237.65 ± 74.87 g.

Isostichopus sp. aff *badionotus* is a gonochoric species that lacks external sexual dimorphism; the gonad has a bifurcated attachment to the dorsal mesentery, located on either side at the front of the body (Fig. 3). Gonads are organized by numerous branched tubules that group into a single duct that opens by the gonopore located in the anterior dorsal portion of the body.

Gonad sizes showed a gradual increase and the cell stages distribution was linked to the degree of development (Figs. 4a and b). During the reproductive cycle, some differences in colour and size of gonadal tubules for both sexes were found. Female gonads always presented a beige-yellow colouration and tubules were larger, reaching a diameter of 405.07 ± 248.19 μm (Figs. 5a and b), while males exhibited a whitish colour and thinner tubules with a diameter of around of 272.18 ± 56.37 μm (Figs. 5c and d). In most months, these characteristics were permanent in almost all samples, although sometimes two colours were apparent, allowing identification of gonadal development status, which also was corroborated by histological analysis.

From the 120 individuals examined 51.3% were male, 43.3% were females and 5.4% could not be differentiated because they had no gonads. The male:female ratio varied during the sampling

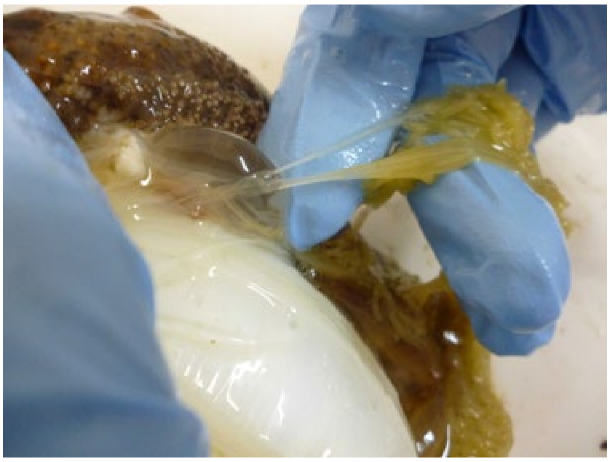

Figure 3. Bifurcated gonad on the *Isostichopus* sp. aff *badionotus* mesentery.

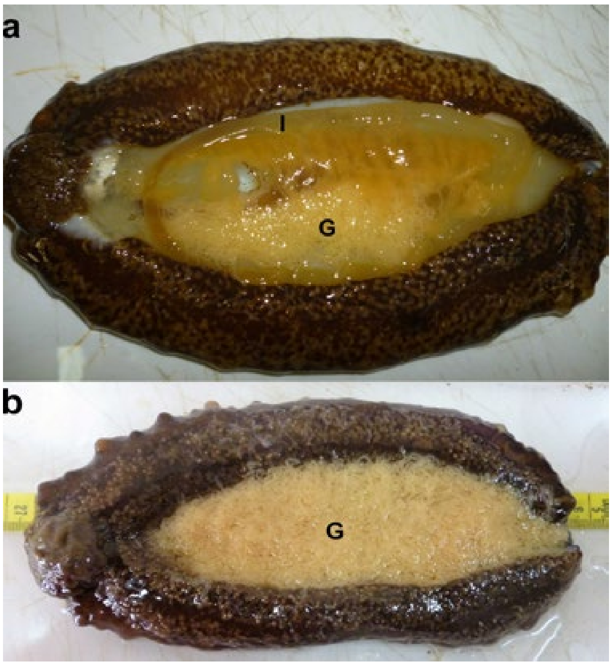

Figure 4. Gradual increase in female gonad size: a) gonad in growth (G) and differentiated intestine (I); and b) fully mature gonad.

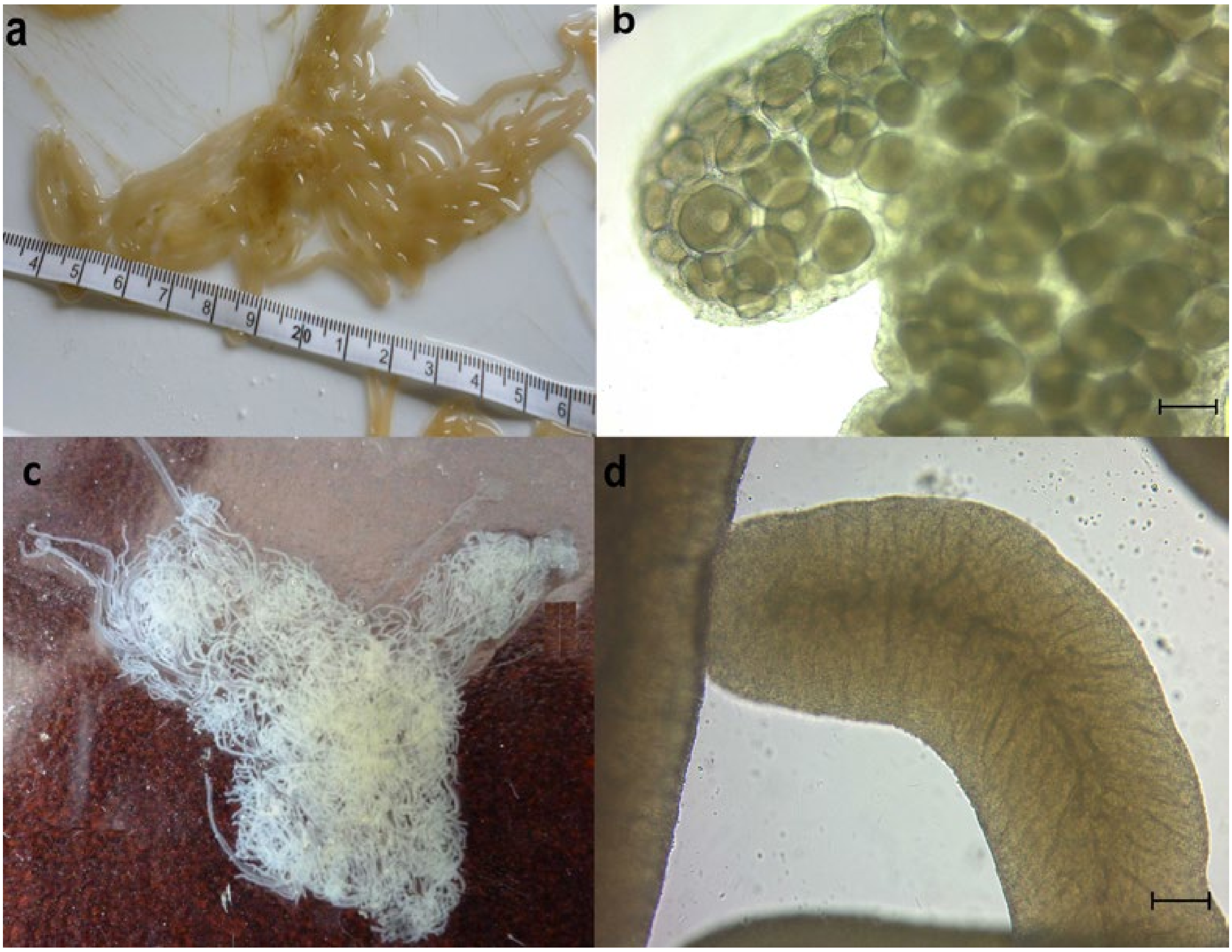

Figure 5. *Isostichopus* sp. aff *badionotus*. Gonads – a) macroscopic view of female gonad that shows tubules with phagocytes (P); b) Light microscopy showing "fresh" oocytes in the process of growth, GV: germinal vesicle; c) macroscopic view of whitish coloured male gonad; d) Light microscopy of "fresh gonadal male" that shows seminiferous channels directed towards the lumen.

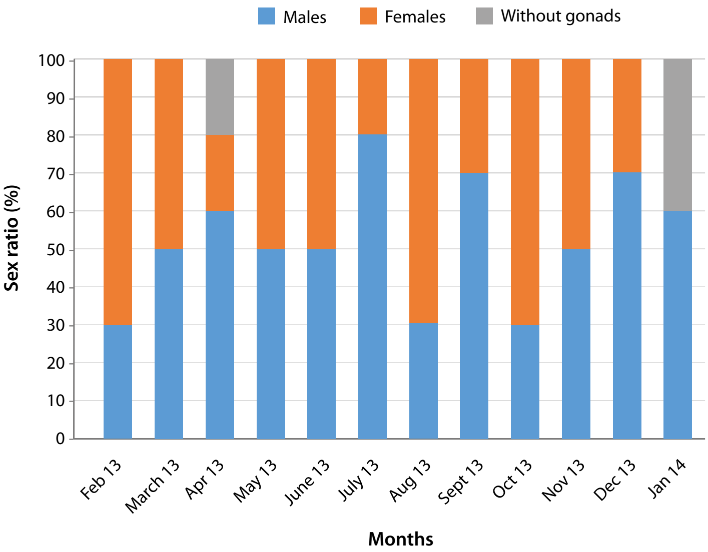

Figure 6. Sex ratio of *Isostichopus* sp. aff *badionotus*. during one year of sampling February 2013 to January 2014. n = 120. ($X2 = 0.314$, $p < 0.05$).

period: in February, August and October the male:female ratio was 1:2.33; during March, May, June and November the ratio remained 1:1; while in April, July, September and December, a greater proportion of males (Fig. 6) was recorded. Total sexual ratio obtained during the sampling was not significantly different from the expected distribution ($x^2 = 0.314$, df = 44, $p < 0.05$); therefore, no difference in sex ratio (1:1) was found.

Description of the reproductive cycle

Gonadosomatic index

The maximum values of GI were observed when the gonad was ripe; while in the resting phase and post spawning phase, lower values were observed due to the reduction in size. Higher values of GI were observed from July to November. The reproductive peak was recorded in October with an average value of 8.72 ± 4.4. Statistical analysis for months showed significant differences during the study ($p < 0.05$), and a relationship between August and November was evident (Fig. 7).

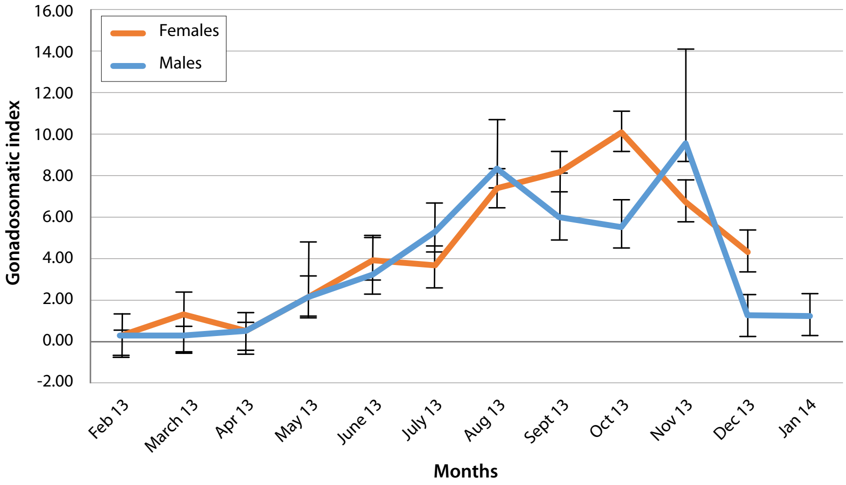

Figure 7. Monthly gonadosomatic index of *Isostichopus* sp. aff *badionotus*, with standard deviation (SD) from February 2013 to January 2014. n = 120. (X2 = 0.314, P < 0.05).

In the analysis by sex, GI showed a similar trend. Males showed GI values of 0.08–16.17, while females showed GI values in the range of 0.06–18.48. When analysing the values based on indicators by gender, no significant differences ($p < 0.05$) between males and females were found, indicating synchronous gonadal development.

The reproductive cycle began in February (2013), when GI values were the lowest. In July, a significant GI increase signalled the onset of ripening. This trend continued for females until October when GI began to decrease rapidly, while males showed a decrease during September and October but GI increased again in November and then declined dramatically from December to January (Fig. 7).

Gonadal histology

Histological analysis of the gonads and gonadosomatic index showed an annual pattern of gonadal development for both sexes corresponding to a complete cycle. Based on the morphological and histological characteristics, the gonadal development for females and males were identified and described as follows:

Oogenesis

In females, five stages of gonadal development were observed: recovery, growth, mature, partially spawned and postspawning (Fig. 8).

Recovery. Gonadal tubule wall is thick and lined by an outer or coelomic epithelium. In this study this structure was found in most stages of gonadal development. Immature oocytes at the periphery of the gonad were observed, delineated by a germ layer that also presented a basophil cytoplasm and nucleus with peripheral nucleoli. The average size of these oocytes was 30.14 ± 10.9 µm. This state was found during February.

Growth. The thickness of the connective tissue that runs along the gonadal tubules becomes thinner. In the germinal epithelium, few immature oocytes were observed, and in the centre of the gonadal tubule, abundant basophilic oocytes at the vitellogenic and previtellogenic stages can be seen. In the cell tissue, we observed a large basophilic cytoplasm and eosinophil nucleus, in which was located a peripheral nucleolus. The average size of these oocytes was 70.5 ± 27.92 µm. This stage was present from March to July.

Mature. Wide tubules were observed. Gonadal tissue showed a reduced wall thickness, making the cœlomic epithelium almost imperceptible. The tubules were completely filled with mature oocytes, formed by an abundant eosinophilic cytoplasm, a large nucleus and nucleolus located in the central or peripheral position, supported on a thin layer of connective tissue. On the periphery of the gonad (germinal epithelium), few immature oocytes were apparent. The average size of mature oocytes was 125.24 ± 13.11 µm. This stage of development was found from June to November.

Partially spawned. Gonadal tubules were observed with abundant mature oocytes, interspersed with empty areas that show the expulsion of the oocyte during spawning. This stage was established before the appearance of phagocytes, which feed waste oocytes. This condition is most common during the months of spawning (August to December).

Post-spawning. The tubular wall was thin and composed of connective tissue, surrounded by squamous cell and a clear coelomic epithelium. Inside the structure, few basophils residual oocytes were

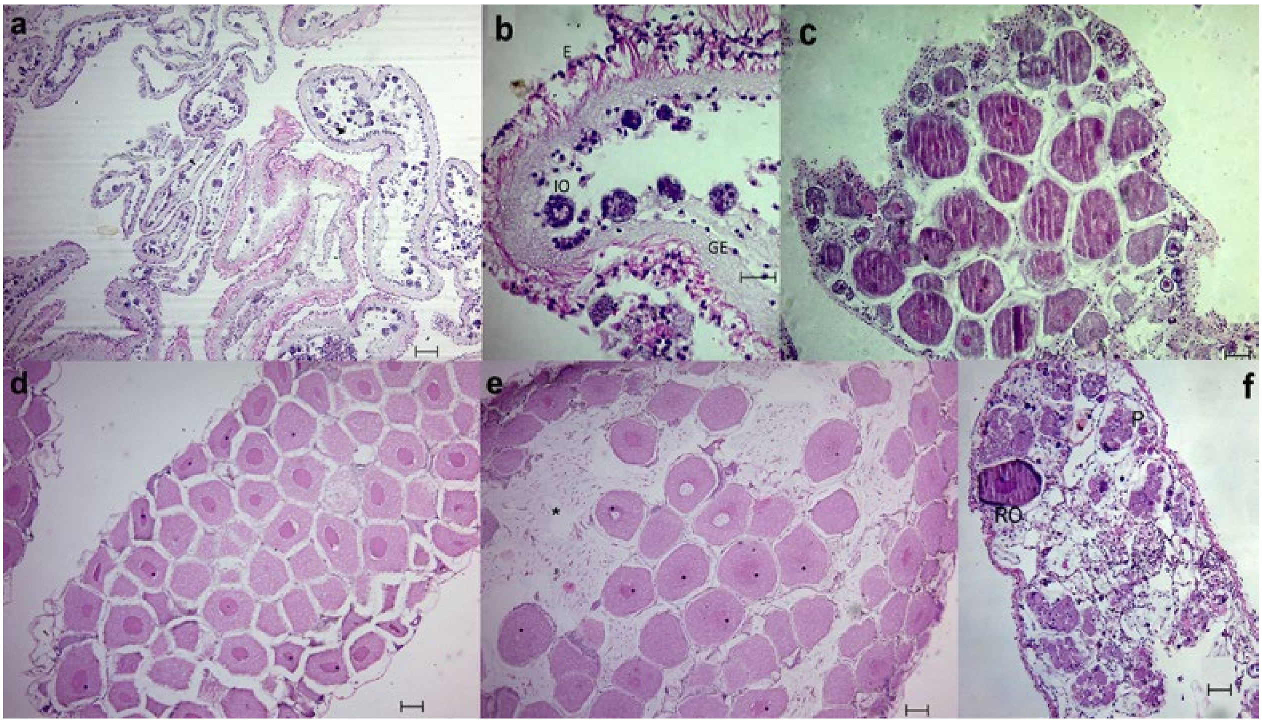

Figure 8. Photomicrography of female gonad development of *Isostichopus* sp. aff *badionotus*. a) Recovery 4x; b) Recovery: outside epithelium (E); Germinal epithelium; immature basophils oocytes. 40x; c) Growth: abundant immature oocytes and other states of previtellogenic development and vitellogenic ovocites, markedly eosinophils. 10x; d) Mature: abundant previtellogenic oocytes and vitellogenic oocytes, containing a large germinal vesicle are seen 10x. e). Partly spawned. Displays empty follicles (*) and vitellogenic oocytes with germinal vesicle and a peripheral nucleoli; f) Postspawning: A damaged tissue with residual basophils oocytes and the presence of abundant phagocytes can be seen (February to December 2013). The scale bar equals 50 µm.

observed, which have different degrees of deterioration; at this stage, phagocytes appear within the area bounded by the remains of the vitelline membrane surrounding the oocyte. This stage was found in February, August, September and November.

Spermatogenesis

Male gonads were characterised by four stages of gonadal development: recovery, growth, mature and post spawning (Fig. 9).

Recovery. The tubular wall was thick, comprising a thin layer of connective tissue with abundant germinal tissue layers on which primary sperm cells are generated. These cells were basophilic, circular in shape and uniform in size. The tubular lumen was empty and no sperm was apparent.

Growth. A thin layer of connective tissue that surrounds the spermatic tubules was observed. Abundant sperm cells (spermatids, spermatocytes) were apparent, organised in a centripetal direction (i.e. from the periphery toward the lumen and then, in like a labyrinth (Fig. 9b).

Mature. Small cells were located in the centripetal position in the tubular lumen, which were identified as mature sperm. The tubular wall was smooth, and in its periphery were numerous spermatocytes.

Post-spawning. The lumen of the tubule was virtually empty and free of sperm. The wall of the tubule showed some invaginations, typical of the germinal epithelium, and the route of sperm was evidenced by the lumen outward.

Gonadal development showed an asynchrony between sexes.

Most females during February were in “post-spawning”, while a slight portion (33.4%) was in “recovery”. In March, gonads in “growth” were observed and this stage was dominant in all individuals sampled over the next two months. During June, July and August, females with “growth” ovaries were observed, indicating the beginning of the reproductive period. In June, there was a high proportion of females in the “mature” stage (60%) accompanied by females in the “growth” stage. A similar pattern was observed during July with a 1:1 ratio between “mature” and “growth”. In August, “partially spawned” (50%), “post-spawning” (16.7%) and “mature” (33.3%) females were seen. “Post-spawning” and “partially spawned” sea cucumbers were found only in September. In October, once again,

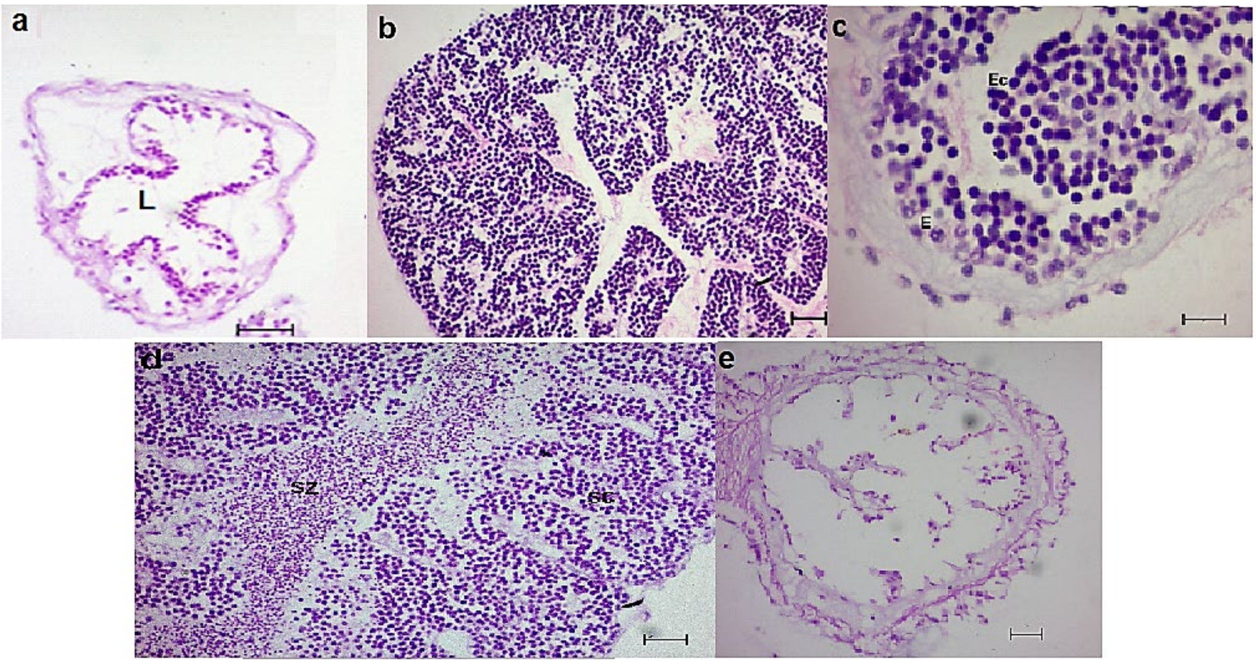

Figure 9. Photomicrography of histology of gonadal development of male *Isostichopus* sp. aff *badionotus*. a) Recovery. A thick layer of connective tissue and basophilic germinal tissue layers is observed. Lumen is empty. 40x; b) Growth. Thin layer of connective tissue surrounding the spermatic tubules (40x), with abundant spermatogonia and spermatocytes, more apparent in photo c). 100x. d) Mature. Sperm in the tubular lumen ready for release. Abundant follicles in the seminal spermatocytes are observed. 10x. e) Postspawning. Seminal follicle is empty. Germinal epithelium tubular wall. 40x. (February 2013 to January 2014). The scale bar equals 20 µm.

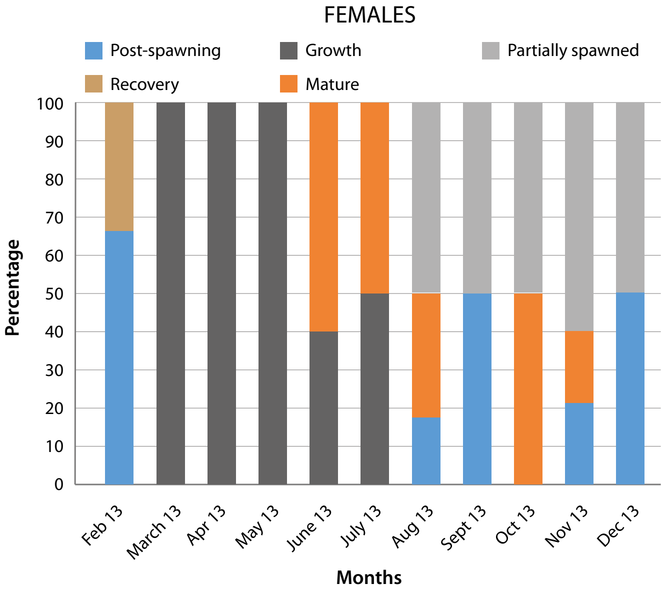

Figure 10. Frequency of gonadal development of female *Isostichopus* sp. aff *badionotus* from February 2013 to December 2013. n = 120.

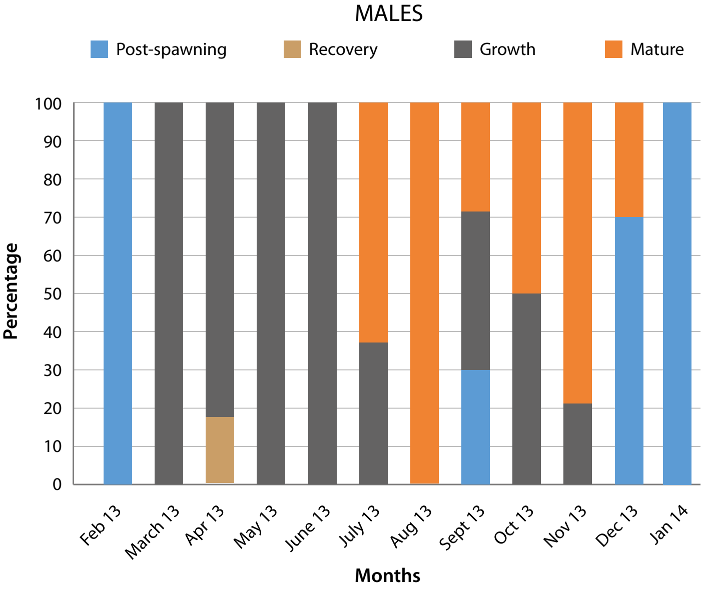

Figure 11. Frequency of gonadal development of male *Isostichopus* sp. aff *badionotus* during February 2013 to January 2014. n = 120.

"mature" and "partially spawned" individuals were present in equal proportion (50%). In November, "partially spawned" (60%), "mature" (20%) and "post-spawning" (20%) stages were identified. In December, the same conditions as in September were observed (Fig. 10).

Males showed two reproductive periods as evidenced by the presence of mature individuals from March to May and July to November. August was the reproductive peak month, with 100% of mature individuals. Males were found to be 100% "post-spawning" only in February 2013 and January 2014. "Growth" and "mature" males were observed almost every month and were present in 100% of the individuals sampled in June. "Recovery" was only found in April (16.7%) (Fig. 11).

Spawning and larval development

Several spawnings were obtained with 4.9 x 10^6 and 6.4 x 10^6 of fertilised eggs produced each month from August to November, at night and dawn. Most of these spawnings took place during the new moon phase (80%). Males begin by releasing sperm followed by females spawning. That behaviour was consistent every year during spawning season.

Fertilisation characteristics were noticeable by the formation of the membrane around the fertilised oocytes. Figure 12 shows different phases of *Isostichopus* sp. aff *badionotus* with embryonic development, from fertilised eggs to gastrula, and then from early auricularia until the doliolaria stage. The development lasted for 28–30 days up to the doliolaria stage at 26°C. In 2014, larval development reached the late auricularia stage, while in 2015 it reached the doliolaria stage (Table 1).

Table 1. Development of *Isostichopus* sp. aff *badionotus* from fertilisation to the doliolaria stage at 26°C. n =40 samples in each stage.

	Size (µm)	
Larval stage	**2014**	**2015**
Fertilised egg	138.02–175.05	146.45–187.75
Blastula	262.72–302.79	286.45–354.67
Gastrula	278.40–356.30	298.34–376.56
Early auricularia	425.30–476.50	496.43–563.93
Mid auricularia	609.28–788.83	576.58–663.07
Late auricularia	787.59–1087.66	976.01–1045.01
Doliolaria		726.98–765.08

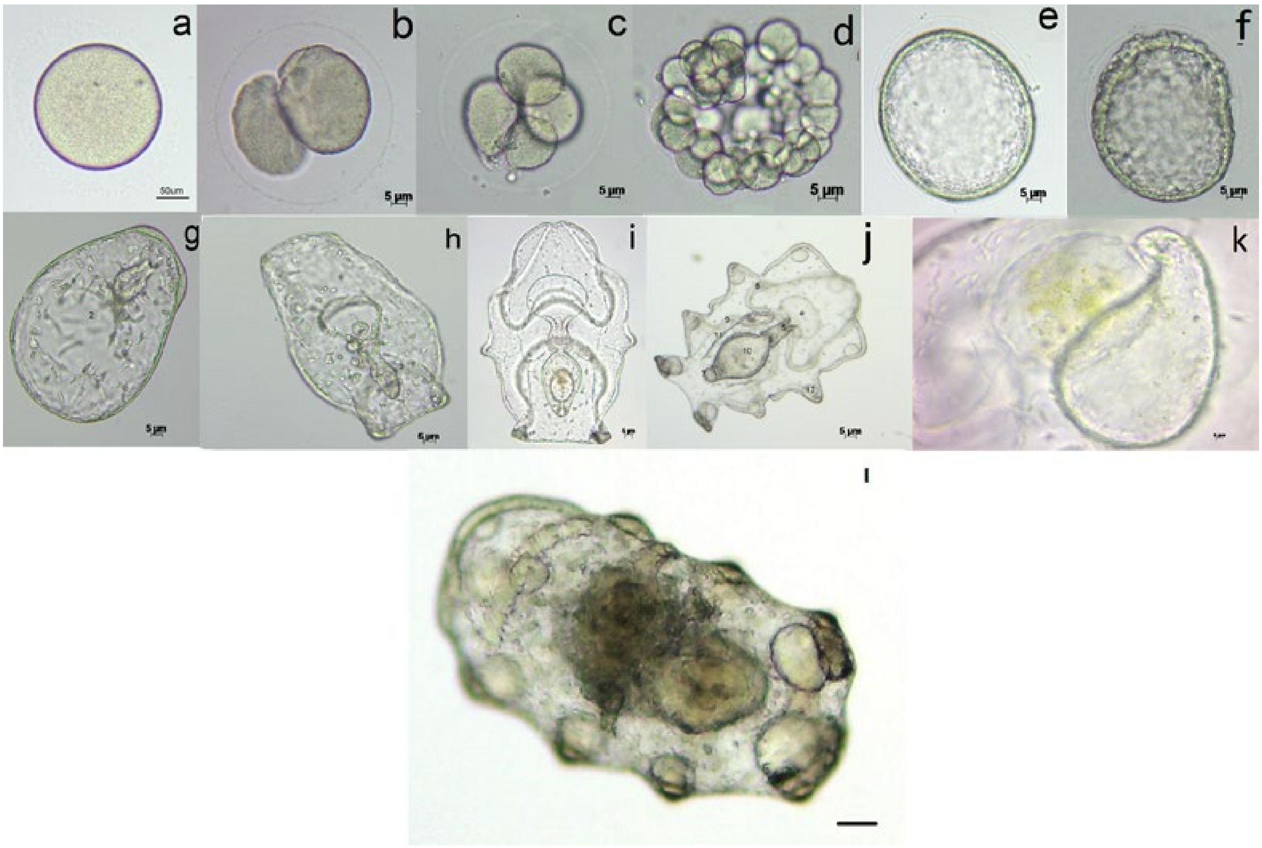

Figure 12. Embryonic and larval development of *Isostichopus* sp. aff *badionotus*.
a) Fertilised egg; b–d) Cleavage stages; e) Early blastula; f) Late blastula; g) Gastrula – (1) archenteron, (2) mesenchyme cells; h) Early auricularia – (3) dorsal pore; i–j) Auricularia – (4) buccal ciliated cavity, (5) oesophagos, (6) stomach, (7) cloaca, (8) ventral pre-oral band, (9) ventral post-oral band, (10) Intestine, (11) Anxohydrocoel (axo), (12) Hyaline sphere; k) Digestive tract with food; l) Doliolaria.

Discussion

Gametogenesis

The sex ratio estimated for *Isostichopus* sp. *nov.* did not differ significantly from 1:1, coinciding with what has been reported for *I. badionotus* and *Holothuria mexicana* (Guzmán et al. 2003), and *H. spinifera* (Asha and Muthiah 2008). Several studies report that this ratio is characteristic of holothurid species with sexual reproduction (Conand 1993; Herrero-Perezrul et al. 1999). The tubules were present in most individuals sampled throughout the year and were not absorbed after spawning; the absence of tubules were evidenced in only five individuals representing 4.16% of the total sampled population. Studies carried out by Herrero-Pérezrul et al. (1999) argue that the absence of gonads can be associated with the evisceration processes, but in our case, sea cucumbers did not show any evidence of evisceration as all organs were present (intestine and respiratory tree), except gonads. During the histological monitoring of gonadal development, the stage of resorption was not recorded. The same situation has been reported for other species of sea cucumbers such as *Holothuria leucospilota* (Ong Che 1990), and *H. fuscogilva* and *Actinopyga mauritiana* (Ramofafia et al. 2000). Some authors, such as Hamel et al. (1993), suggest that the presence of phagocytes inhibits tubular reabsorption of gonads; for this reason, the tubule recruitment model proposed by Smiley (1988) may not apply to *Isostichopus* sp. aff *badionotus*.

For female *Isostichopus* sp. aff *badionotus*, five stages were described; whereas in males only four stages were described. The fluctuation of GI during the sampling period revealed a significant increase from July to November, with a peak in October, indicating that *Isostichopus* sp. *nov.* is a species with an annual reproductive cycle, which presents a single spawning event during the warmer months of the year (Agudelo-Martinez and Rodríguez-Forero 2015). Smiley et al. (1991) considered that this reproductive preference for the warmer months is related to sea temperature and food availability for planktonic larvae development. The same situation was reported for *I. badionotus* (Herrero-Pérezrul et al. 1999; Foglietta et al. 2004; Zacarías-Soto et al. 2013). This situation is also common to most aspidochirotides of temperate and tropical zones (Hyman 1955; Smiley et al. 1991; Zacarías-Soto et al. 2013; Agudelo and Rodríguez 2015).

Spawning and larval development

Spawning season was linked to the increase in GI from July until November. During December to June natural spawning was not viable. Our results are in agreement with those obtained for *Isostichopus badionotus* in the tropical western Atlantic (Guzmán et al. 2003; Foglietta et al. 2004; Zacarias-Soto et al. 2013). It is well known that moon phases play an important role in the onset of spawning in holothuroids (Babcock et al. 1992; Kubota and Tomari 1998; Morgan 2000; Battaglene et al. 2002; Guzmán et al. 2003; Hu et al. 2010); this study showed that is also the case for *Isostichopus* sp. aff *badionotus*.

Embryonic and larval development of *Isostichopus* sp. aff *badionotus* was similar to other tropical sea cucumbers, showing the same phases: blastula, gastrula, auricularia and doliolaria.

Commercial value

Isostichopus badionotus is one of the sea cucumber species collected by a large artisanal and semi-industrial sea cucumber fisheries established in Colombia for several years. It is possible that most of the sea cucumber catches reported as *I. badionotus* were in fact *Isostichopus* sp. aff *badionotus*. Export statistics to Asian countries would, therefore, be wrong. In 2013, sea cucumbers were sold by artisanal fishers at low prices (USD 0.5 unit^{-1}, or USD 1–3 tkg^{-1}) (Rodríguez et al. 2013). Currently, commercial sea cucumber fisheries are illegal in Colombia. Considering that *Isostichopus* sp. aff *badionotus* has a healthy chemical composition similar to that of sea cucumbers internationally traded, it could be a species with a competitive commercial future (Vergara and Rodríguez 2016), and recent progress with its aquaculture (Agudelo and Rodríguez 2015) could make it a suitable candidate for the international market.

Acknowledgements

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New host for the parasitic worm *Anoplodium* sp. (Rhabditophora: Platyhelminthes) found in the sea cucumber *Isostichopus fuscus* (Holothuroidea: Echinodermata)

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Abstract

A flatworm was discovered inside the coelomic cavity of the commercial sea cucumber *Isostichopus fuscus* along the Pacific coast of Mexico. Based on morphological and genetic evidence, it was determined to be *Anoplodium* sp. belonging to class Rhabditophora. Thus, the sea cucumber *I. fuscus* constitutes a new host. The flatworms were consistently found on the surface of the haemal vessels and the *rete mirabile* of 92% of the sea cucumbers sampled along the cost of Mazatlan, and 88% of the sea cucumbers collected in the Sea of Cortez. The infestation rate varied from 1 to 725 flatworms per individual, in both male and female sea cucumbers. When more than ~120 *Anoplodium* sp. were counted in a single host, the gonads of the latter were either very small (≤1.2 g wet weight, or GI <0.26) or absent, suggesting that the flatworm could be detrimental to *I. fuscus* and be considered parasitic. Combined with the threat of overfishing throughout its distribution range, the discovery of this parasite could seal the fate of *I. fuscus* in certain regions of the eastern Pacific.

Introduction

Sea cucumbers are known to host a variety of associated species that may dwell externally — on their body wall, around their mouth, and among their tentacles — or internally, inside the respiratory tree, intestines, or coelomic cavity (Jangoux 1987; Eeckhaut et al. 2004). In particular, sea cucumbers living in the Indo-Pacific and the Caribbean regions can host several species at a time and sometimes in considerable numbers. Crustaceans, gastropods, bivalves, and fishes are described either as epibionts or endobionts, and are considered to be either obligate or facultative associates of sea cucumbers. These associations are mostly determined to be commensal (e.g. Mercier and Hamel 2005) or parasitic (e.g. Heading and Mandahl-Barth 1938; Hamel et al. 1999). Over a decade ago, Eeckhaut et al. (2004) indicated that ~150 species of metazoans could be found in sea cucumbers, and this number is increasing every year.

Worms are commonly reported in association with sea cucumbers (Jangoux 1987; Eeckhaut et al. 2004), including members of classes Rhabditophora (e.g. *Anoplodium*, *Avagina*, *Cleistogomia*, *Syndesmis*), Trematoda (e.g. *Fellodistomun*, *Monorchis*, *Zoogonus*), Nematoda (*Ananus*, *Onchaleimus*, *Thalassonema*) and Polychaeta (e.g. *Ophryotrocha*, *Gastrolepia*). Among the associates of echinoderms, Jangoux (1987, 1990) described 58 Rhabdocoela.

Within the phylum Platyhelminthes, members of the order Rhabdocoela, and especially the genus *Anoplodium*, have been reported from various species of sea cucumbers spreading from polar to tropical habitats (e.g. Marcus 1949; Westblad 1953; Hyman 1960; Doignon et al. 2001). The number of associates found in each host varied between 1 and 202. For example, Doignon et al. (2001) found between 128 and 202 *Anoplodium parasitica* in the sea cucumber *Holothuria tubulosa*. The same species was also found to parasitise *H. polii* and *H. stellati*. These worms typically infest the coelomic cavity or the digestive tract of their hosts (Jangoux 1987). The species *Anoplodium hymanae* described by Shinn (1983, 1985a, b) can be found in the coelomic cavity of 88–94% of *Stichopus californicus* on the west coast of North America (Washington State, USA), with an average prevalence of 15 worms per sea cucumber. Shinn (1983) considers *Anoplodium hymanae* to be parasitic because it was found to consume a large number of host coelomocytes. Shinn (1985a, b) also described the reproduction and infestation mechanisms of the species.

The present work outlines a new host for a species of the genus *Anoplodium*, reports on its infestation

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rates, and discusses negative impacts on the host, supporting its classification as parasitic.

Materials and methods

Collection

Specimens of the sea cucumber *Isostichopus fuscus* were collected by divers along the coast of Mexico in the Sea of Cortez (25.59N: 110.88W; n=430 individuals) and off the coast of Mazatlan in western Mexico (23.27N: 106.63W; n=734 individuals) in June and July 2009 between 5 and 15 m depth.

Out of those, 45 sea cucumbers were analysed from the Sea of Cortez and 112 from Mazatlan. The contracted length (mouth–anus), whole wet weight and eviscerated body wall wet weight of each sea cucumber was measured. Sex was determined from a biopsy of the gonad and gamete smears examined under a light microscope. The gonad index was established as the percent wet weight of the gonads on the eviscerated body wall wet weight. The haemal system and the respiratory tree were carefully transferred to a Petri dish, and the coelomic cavity and the surface of the intestines were rinsed with filtered seawater. The flatworms present were counted and measured. Because individuals are slightly oval in shape (Fig. 1), two measurements were taken, one of the longest axis and the other of the axis perpendicular to it. Subsamples of the flatworms were preserved either in 100% ethanol (n = 300–350) for genetic analyses, or 4% formaldehyde (n~200) for histology and morphological description.

Histology and DNA extraction and sequencing

To identify and characterise the worm species, two different approaches were used. First, histological slides were prepared from preserved samples. Five formaldehyde-fixed samples and 5 ethanol-preserved samples were immersed in a 3% solution of glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8) for 3 h at 4°C. They were then rinsed in the buffer and post-fixed for 1 h in a 1% solution of osmium tetroxide in the same buffer. After a final rinsing in the buffer, they were dehydrated in a graded ethanol series and embedded in Spurr resin. Serial transverse and longitudinal 1 µm thick sections were made with a glass knife using a Reichert Om U2 ultramicrotome, and stained in a 1:1 solution of methylene blue and azur II according to the method used by Humphrey and Pittman (1974).

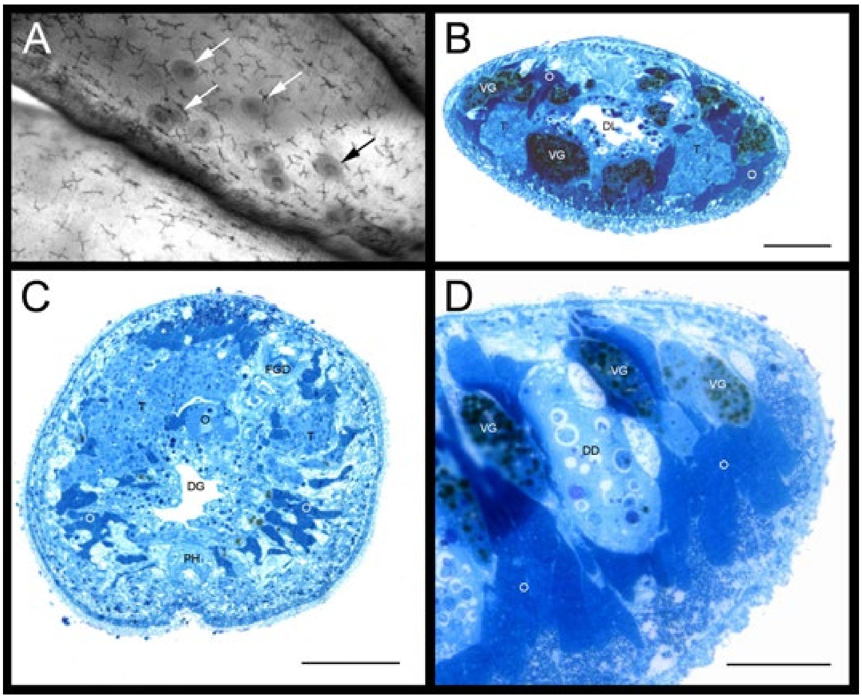

Figure 1. A. Live individuals (500–700 µm) of the worm *Anoplodium* sp. (arrows) on the *rete mirabile* of the sea cucumber *Isostichopus fuscus*. B. Transverse section in the middle part of the body. C. Frontal section in the ventral part of an individual. D. Details of the internal tissues (longitudinal section). Scale bar in B and C represents 100 µm, scale bar in D represents 50 µm. DD = digestive diverticula; DL = digestive lumen; FGD = female genital ducts; O = ovary; PH = pharynx; T = testicle; VG = vitelline gland.

Secondly, identification was made using 18S rDNA sequencing. Five ethanol-preserved samples were pooled together, and their genomic DNA extracted with the commercial kit Invitek Spin Tissue Mini kit (Invisorb). DNA fragments from the 18S rDNA (ca. 1,200 nucleotides in all) were amplified by a polymerase chain reaction and sequenced following the detailed procedure described in Eeckhaut et al. (2000) and Lanterbecq et al. (2006). The sequences obtained were processed and cleaned using the program code Codon Align, and subjected to a BLAST (Basic Local Alignment Search Tool) search in the National Center for Biotechnology Information database. Two portions of the 18S rDNA of ca. 600 bp each were deposited in Genbank under accession numbers KY320456 and KY320457.

Results and discussion

Species identification

Based on morphological characteristics, the flatworm that was found to infest the commercial sea cucumber *Isostichopus fuscus* in both locations along the coast of Mexico was identified as *Anoplodium* sp. (Platyhelminthes: Rhabdocoela). Two 18S rDNA fragments of 640 and 555 p long were obtained and checked against the BLAST database, which confirmed that the species belonged to the genus *Anoplodium*, with 95% and 93% similarity to *Anoplodium stichopi* (Table 1).

Table 1. The seven closest related species according to BLAST searches on the two 18S rDNA fragments of the parasite, with the percentage of identity of the fragments, and the accession numbers of the related sequences.

Most related species	Fragment 1	Fragment 2	Accession number
Anoplodium stichopi	96	94	AF167424.1
Seritia elegens	93	92	KC529517.1
Wahlia macrostilifera	92	91	KC529518.1
Veidovskya ignava	90	87	KC529513.1
Veidovskya pellucida	90	87	KC529512.1
Provortex balticus	92	86	KC529511.1
Provortex karingi	92	85	KC529510.1

However, the latter species is from the Mediterranean Sea. Sequences from *A. hymanae (US West Coast)* are not available for optimum comparison with Shinn (1983). However, the morphology of the specimens found here differs markedly from previous descriptions, suggesting a new species of *Anoplodium*. Specimens were deposited at the Department of Ocean Sciences, Memorial University (Canada).

The flatworm's body is flat and longer than it is wide, with the anterior end slightly truncated. The samples were characterised by two main size classes. The largest specimens were between 750 ± 75 µm long and 690 ± 55 µm wide (comprising ~29% of the flatworms collected) and the smallest individuals were 590 ± 25 µm long and 500 ± 25 µm wide (Fig. 1) comprising the majority of individuals. Compared with the maximum size of *A. hymanae* described by Shinn (1983), which were 2.0–2.5 mm long, specimens in the present study are clearly smaller. Live specimens were white or greyish and the body wall was transparent (Fig. 1a). Histological sections showed a uniform ciliated epidermis, a blind digestive system with caeca and a pharynx opening ventrally, and two testicles extending along both sides of the body. The main elements of the female genital systems observed on sections were the vitelline glands and what we suppose are two ovaries that also extend along both sides of the body.

Prevalence and infestation rates

Between 88% and 92% of all sea cucumbers from Mexico hosted the Rhabdocoela *Anoplodium* sp. in the present study, exhibiting prevalence rates similar to those reported by Shinn (1983) for *A. hymanae* in *Stichopus californicus* (89–94%). However, the prevalence rate is high relative to other infestation rates recorded for the same genus; for example, 13% of *Holothuria tubulosa* were reportedly infested by *A. parasitica* in the Mediterranean Sea (Doignon et al. 2001). Furthermore, a maximum of ~750 flatworms per host were found in the present study, much more than the 15 flatworms described in *S. califiornicus* by Shinn (1983). Infestation rates of ~200 flatworms per host were found in some tropical sea cucumbers from Papua New Guinea (Eeckhaut et al. 2004). Overall, the present study represents the highest number of *Anoplodium* per host ever observed in a sea cucumber (Jangoux 1987). The extreme case of flatworm infestation recorded here could be attributed to the fact that *I. fuscus* at both locations occurred in highly populated and polluted areas along the coast (e.g. sewage discharge). The water quality could have stimulated the proliferation of coelomocytes and indirectly lead to favourable food sources for the parasites, thereby enhancing their multiplication.

Shinn (1983, 1985a, b) reported that that *A. hymanae* competed with its host for nutrients and thus may elicit adverse side effects. He demonstrated that the flatworms could consume coelomocytes in *Stichopus californicus*, and concluded that *A. hymanae* was indeed parasitic. The present study showed a drop in the host's gonad size (≤1.2 g wet weight) when the number of *Anoplodium* sp. reached ≥120 individuals per host. This castrating effect was recorded in both male and female sea cucumbers. Non-infested sea cucumbers displayed gonad index values between 6.3 and 12.4 compared with a maximum of 0.24 in infested individuals. An overabundance of flatworms in the coelomic cavity may deplete the coelomocytes to the point of preventing gonad growth, supporting the classification of *Anoplodium* sp. as a parasite of the sea cucumber *I. fuscus*.

Effect on host and conservation concerns

In the present study, *Anoplodium* sp. was found to occur on the surface of the haemal vessels and the *rete mirabile*. Individuals occurred mainly in small groups of 3 to 12 and were sometimes overlapping each other (Fig. 1a). When removed from the coelomic cavity of the sea cucumber host, *Anoplodium* sp. did not survive more than ~2 h in ambient seawater.

Anoplodium sp. colonised both male and female sea cucumbers.

The sea cucumber *Isostichopus fuscus* is the second most expensive sea cucumber on the market, just after *Apostichopus japonicus*; it is fished throughout its distribution range and has been listed as endangered on the International Union for Conservation of Nature Red List (Mercier et al. 2013). Therefore, the presence of the parasite *Anoplodium* sp. could exacerbate the threat of overfishing in certain areas of the eastern Pacific, through a significant decrease in reproductive output. This finding suggests that the presence of *Anoplodium* sp. in fished populations of *I. fuscus* should be monitored closely as part of the management plan; otherwise, the species could suffer local extinctions in the near future, at least in heavily infested areas along the coast of Mexico. Future investigations should seek to determine whether this parasite occurs in other areas of the distribution range of *I. fuscus*, which extends as far south as Ecuador.

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Symbionts of the giant red sea cucumber, *Parastichopus californicus* (Stimpson, 1857), with some implications for culture of the host

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Abstract

Commercial fishing impacts on giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) populations in Washington State, USA have prompted the development of hatchery methods for and commercial-scale aquaculture of the species. As these practices grow, it will become increasingly important for culturists to be familiar with naturally occurring *P. californicus* symbionts. For the first time, all of the known symbionts of the giant red sea cucumber are briefly reviewed. Furthermore, new data are presented concerning the ecology of a commensal scaleworm and an endoparasitic snail. This information can be used by culturists and hatchery workers when establishing health management plans for and identifying possible triggers of harmful conditions affecting reared *P. californicus*.

Introduction

The giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) is fully exploited in shallow (depth ≤ 36 m relative to mean lower low water), inland marine waters of Washington State in the United States of America (USA) (Carson et al. 2016; Mueller 2016). In fact, commercial fisheries have resulted in local depletions of *P. californicus* in some management areas of the state, prompting natural resource authorities to develop aquaculture methods and stock enhancement plans for the species (PSI undated; Williams 2014). While scientists have pursued experimental aquaculture of *P. californicus* in Alaska, USA and British Columbia (BC), Canada, mostly in conjunction with other marine species (Ahlgren 1998; Paltzat et al. 2008; Hannah et al. 2013), it has not been until recently that researchers have demonstrated the plausibility of commercial-scale aquaculture of the giant red sea cucumber (DFO 2014; Royal 2016; Suhrbier et al. 2016). As the practice develops beyond experimentation, it will become increasingly important for culturists to be aware of the natural occurrence of *P. californicus* symbionts (Yanong and Erlacher-Reid 2012; Blaylock and Bullard 2014; Overstreet and Lotz 2016), especially because some symbionts have been problematic for cultured sea cucumbers elsewhere (Eeckhaut et al. 2004; Shinn et al. 2015). For the purposes of this paper, the definition of the symbiotic relationship between the giant red sea cucumber and its associated species will follow Lincoln et al. (1992); i.e. all relationships and interactions between members of two different species, including commensalism, mutualism, parasitism, and other relationships (e.g. bacterial), which has been used similarly by others (Overstreet and Lotz 2016). Endosymbionts are referred to as *infecting* a host, whereas ectosymbionts *infest* a host (Overstreet and Lotz 2016). General terms and quantitative descriptors of symbiont populations (e.g. *prevalence* or *intensity* of), especially those of parasites, follow Bush et al. (1997). Following is the first (albeit brief) comprehensive review of the associations between *P. californicus* and its known symbionts. In addition, novel information is provided concerning the ecology of two rather charismatic symbionts of the giant red sea cucumber. In the end, some implications of these relationships are discussed for the benefit of sea cucumber culturists and hatchery personnel.

Review of giant red sea cucumber symbionts

Bacteria

Until recently, reports of bacterial infections in wild and cultured sea cucumbers have been rare in the scientific literature (Jangoux 1987a; Eeckhaut et al. 2004; Wang et al. 2004; Deng et al. 2009; Liu et al. 2010). There is no documentation of a natural occurrence of bacteriosis in *P. californicus* (DFO 2014); however, the immune response of the giant red sea cucumber has been well documented for nearly 100 years. For example, an early description of *P. californicus* coelomocyte form and function was provided by Kindred (1924). Boolootian and Giese (1958) confirmed Kindred's (1924) findings and added to the number and type of coelomocytes involved in the *P. californicus* immune response. Conversely, Hetzel (1963) reexamined the coelomic structures reported by the others, refined their descriptions of number, form and function, and hypothesized (correctly) that

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holothurian "brown bodies" were not another type of coelomocyte; rather, the yellowish-brown granules were the product of an immune reaction. Hetzel later went on to describe the formation of brown bodies in *P. californicus* and other sea cucumbers (Hetzel 1965). Dybas and Fankboner (1986) demonstrated the strong immune response of *P. californicus* to a variety of marine bacteria (gut-associated) injected into the coelomic cavity of the sea cucumber, including phagocytosis, the formation and elimination of brown bodies, the degradation of encapsulated material, and the bactericidal activity of the lysosomal enzyme, acid phosphatase. The authors surmised that the antibacterial response in *P. californicus* was a survival mechanism that coincided with the seasonal aestivation process where gut-associated bacteria might be exposed to the sea cucumber. Finally, in studying the coelomoducts overlying the rectum of *P. californicus*, Shinn et al. (1990) suggested that, besides helping maintain hydrostatic pressure within the body, the coelomoducts provided a pathway for brown bodies to exit the coelom, thereby facilitating excretion and the immune response of the giant red sea cucumber.

Nonbacterial symbionts

Like many sea cucumbers, *P. californicus* has a rich assemblage of nonbacterial symbionts — representing several taxonomic groups — associated with it (Jangoux 1987a, b). Table 1 summarizes these nonbacterial symbionts and extends Jangoux's lists (1987a, 1987b) to include protozoans and a crustacean symbiont of the giant red sea cucumber; their classifications follow those recently reported for eugregarines and coccidians (Kopečná et al. 2006; Wakeman and Leander 2012), ciliates (Lynn and Strüder-Kypke 2002; Gao et al. 2012), flatworms (Van Steenkiste et al. 2013), endoparasitic gastropods (Colgan et al. 2007), scale-bearing polychaetes (Struck et al. 2011; Norlinder et al. 2012; Norlinder 2013), and pea crabs (Ng et al. 2008; Tsang et al. 2014; Palacios-Theil et al. 2016). Some nonbacterial symbionts have been documented in the past few years or decades, whereas others have been recognized for at least a century or more (Table 1).

Eugregarines and Coccidians

Protozoan symbionts are common in holothurians (Jangoux 1987a; Eeckhaut et al. 2004); however, information on disease-forming varieties is limited (e.g. Massin et al. 1978). For example, Fankboner and Cameron (1985) described blistering on the viscera of *P. californicus* during its annual aestivation process. The blisters contained masses of sporocysts from unidentified eugregarines; their presence was considered normal for the late visceral atrophy phase. Furthermore, MacCallum et al. (2001) mentioned finding only mature oocysts of an unidentified, apicomplexan coccidian in the respiratory tree and cloaca of a captive specimen of *P. californicus* in British Columbia, Canada. Given that there was no apparent reaction to the oocysts by the host, Bower (2004a) ranked the symbiont as one of negligible regulatory significance to Canadian natural resource authorities, citing that prevention and control of the coccidian would be impractical.

Ciliates

On the other hand, with the advent of commercial-scale aquaculture elsewhere, protozoan ciliates have become potentially problematic for cultured sea cucumbers (Wang et al. 2004; Long et al. 2006). The giant red sea cucumber harbors at least two species of these symbionts (Table 1), including one scuticociliate, *Boveria subcylindrica* (Stevens, 1901), that shares the same genus as a disease-causing agent in Asia (Wang et al. 2004; Long et al. 2006). Stevens (1901) remarked that *B. subcylindrica* and a larger spirotrich ciliate, *Licnophora macfarlandi* (Stevens, 1901; but see expanded description by Balamuth 1941), inhabited the respiratory trees of all but one of the 100+ giant red sea cucumber she examined (prevalence ≥ 99%) from the vicinity of Monterey Bay, California, USA. Furthermore, she observed that the smaller *B. subcylindrica* was readily consumed by the larger *L. macfarlandi*, and that there was interannual variation in the intensities of both species. In a natural state, impacts of the ciliates on *P. californicus* are unknown (Jaeckle and Strathmann 2013). Stevens (1901) concluded that the two ciliates had no or little impact on their host, which was echoed a century later by MacCallum et al. (2001), who discovered unidentified ciliates in two captive specimens of *P. californicus* from BC, Canada. Since there were no apparent reactions to the unidentified ciliates by the host, Bower (2004b) ranked these symbionts to be of negligible regulatory significance to Canadian natural resource authorities as well, stating that prevention and control of them would be impractical, especially given the ubiquity of the unidentified ciliates.

Flatworms

Symbiotic flatworms are widespread in sea cucumbers; *P. californicus* being no exception (Jangoux 1987b). The seminal 1980s work of G. L. Shinn involving the relationships between Northeast Pacific echinoderms and their symbiotic flatworms was reviewed in detail by Jangoux (1987b), so will be only briefly discussed here for the giant red sea cucumber. There are two fully-described umagillid flatworms infecting *P. californicus*: *Anoplodium hymanae* (Shinn 1983) inhabits only the coelom, whereas *Wahlia pulchella* (Kozloff and Shinn 1987) inhabits the intestine (Table 1). The former is considered to be parasitic (Shinn 1983, 1985a, b), whereas the latter's endosymbiotic role is not clearly defined (Shinn

1986; Kozloff and Shinn 1987). Fankboner and Cameron (1985) noted a high intensity of *A. hymanae* in the coelom of the host during its annual aestivation process. A third genus of umagillid flatworm, *Ozametra*, was observed by them, but this was likely Kozloff and Shinn's (1987) *W. pulchella*. All of these authors (but especially Shinn) provide substantial detail on the taxonomy, biology and ecology of these flatworms and their relationships with *P. californicus*, including levels of infections (e.g. seasonal and ontogenetic variation), host reaction to reproductive products of the symbionts (e.g. formation and expulsion of brown bodies encapsulating flatworm eggs), and potential pathways of transmission related to life history processes of the giant red sea cucumber (e.g. feeding, seasonal aestivation, and naturally-occurring evisceration of the host).

Endoparasitic snail

The shell-less, endoparasitic snail, *Enteroxenos parastichopoli* (Tikasingh, 1961) (Fig. 1 B), was likely first observed infecting *P. californicus* more than 120 years ago (Harrington and Griffin 1897), but it would take decades before the eulimid snail was correctly identified as a new symbiont of the holothurian (Tikasingh 1960) (Table 1). Studies of *E. parastichopoli* have primarily focused on histological aspects of the gastropod (Tikasingh 1962) and its systematics (Tikasingh and Pratt 1961; Kincaid 1964). As its name implies, *E. parastichopoli* infects only the giant red sea cucumber (Tikasingh 1961).

Early reports suggest that the symbiont enters the host through the latter's alimentary tract or anus, eventually making its way out through the gut wall, and finally attaching itself to the anterior end (and coelomic side) of the host's intestine where the hermaphroditic snail develops and matures (Tikasingh 1960, 1962, 1962; Kincaid 1964). Presumably, infective stages of the parasite are evacuated from the host when *P. californicus* undergoes aestivation or evisceration (Tikasingh 1962; Kincaid 1964). Neither Tikasingh (1962) nor Kincaid (1964) detected larval *E. parastichopoli* in plankton samples; yet Kincaid (1964) anecdotally described the shelled juvenile of *E. parastichopoli*, comparing his observations with a congener of the symbiont. Canadian researchers (Cameron 1985; Cameron and Fankboner 1989) were the first to attempt an assessment of how widespread *E. parastichopoli* infections might be in juvenile and subadult giant red sea cucumbers (≤ 4 years old), and finally, in Jangoux's (1987b) review of the potential impacts of eulimid parasitism on echinoderm hosts, the author concluded that any ecological consequences for the hosts were limited. Indeed, Tikasingh (1962) suggested that the endoparasitic snail probably derives its nutrition from the coelomic fluid of the host rather than the anterior end of the intestine to which it attaches.

Scaleworm

Perhaps the most widely recognized symbiont of *P. californicus* is the scaleworm *Arctonoe pulchra* (Johnson, 1897) (Fig. 1 A). The two have been inexorably paired since the scaleworm was first identified in the 1890s (Johnson 1897; Pettibone 1953; Kozloff 1996); this is especially true for infestations in the San Juan Islands, Washington State, USA (Pernet 1999) (hereafter, San Juan Islands). *A. pulchra* is commensal also with congeners of *P. californicus* (Dimock and Davenport 1971; Lambert 1986). Early studies of *A. pulchra* focused on its behavior in the presence of the giant red sea cucumber, including the scaleworm's ability to recognize its host (Davenport 1950; Davenport and Hickok 1951; Dimock and Davenport 1971). In fact, Davenport and the others' research on *Arctonoe* spp. provided the foundation for understanding host-commensal relationships among polychaetes (Martin and Britayev 1998). Additional studies focused on the taxonomy (morphological and molecular), anatomy, reproduction and successful culture of *A. pulchra* (Pettibone 1953; Pernet 1998, 1999, 2000). And similar to *E. parastichopoli*, Canadian researchers (Cameron 1985; Cameron and Fankboner 1989)

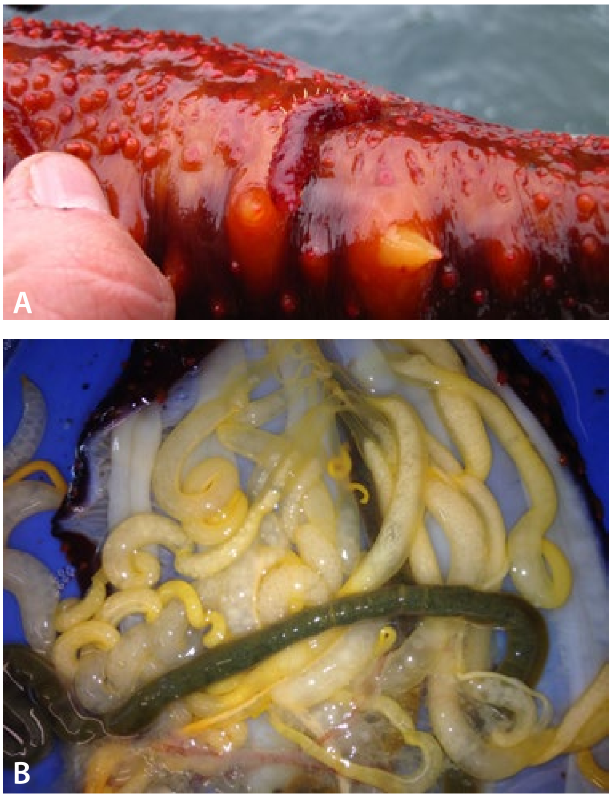

Figure 1. Two symbionts of the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857). A: The scaleworm, *Arctonoe pulchra* (Johnson, 1897), forms a commensal relationship with *P. californicus*. B: The shell-less snail, *Enteroxenos parastichopoli* (Tikasingh, 1961), forms an endoparasitic relationship with its host. Here, numerous yellow, egg-laden *E. parastichopoli* are attached to the anterior portion of the intestine of *P. californicus*.

Table 1. Nonbacterial symbionts of the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) classified to lowest taxonomic rank possible based on their original description in the scientific literature. Taxonomic ranks above genus and species were based on recent molecular phylogenies for those groups of organisms listed (see text for references).

Kingdom	Phylum	Class	Order	Family	Genus and species	Type	Host site	Reference(s)
Protozoa	Apicomplexa	Conoidasida	Eugregarinorida	NA	NA	Unidentifed eugregarine	Viscera	Fankboner and Cameron (1985)
Protozoa	Apicomplexa	Conoidasida	Eucoccidiorida	NA	NA	Unidentified coccidian	Respiratory tree; cloaca	MacCallum et al. (2001)
Protozoa	Ciliophora	Spirotrichea	Licnophorida	Licnophoridae	*Licnophora macfarlandi*	Spirotrich ciliate	Respiratory tree	Stevens (1901); Balamuth (1941)
Protozoa	Ciliophora	Oligo-hymenophorea	Thigmotrichida	Boveriidae	*Boveria subcylindrica*	Scuticociliate	Respiratory tree	Stevens (1901)
Protozoa	Ciliophora	NA	NA	NA	NA	Unidentified ciliates	Respiratory tree	MacCallum et al. (2001)
Animalia	Platyhelminthes	Rhabditophora	Rhabdocoela	Umagillidae	*Anoplodium hymanae*	Flatworm	Coelom	Shinn (1983)
Animalia	Platyhelminthes	Rhabditophora	Rhabdocoela	Umagillidae	*Wahlia pulchella*	Flatworm	Intestine	Kozloff and Shinn (1987)
Animalia	Annelida	Errantia	Phyllodocida	Polynoidae	*Arctonoe pulchra*	Scaleworm	Exterior body wall (i.e. skin)	Johnson (1897)
Animalia	Mollusca	Gastropoda	Sorbeoconcha	Eulimidae	*Enteroxenos parastichopoli*	Endoparasitic snail	Intestine	Harrington and Griffin (1897); Tikasingh (1961)
Animalia	Arthropoda	Malacostraca	Decapoda	Pinnotheridae	*Opisthopus transversus*	Pea crab	Cloaca	Rathbun (1894); Rathbun (1918)

NA = not available.

were the first to attempt an assessment of how widespread *A. pulchra* infestations might be in juvenile *P. californicus*. Lastly, fecal pellet analyses revealed that, while infesting *P. californicus*, *A. pulchra* fed mostly on crustaceans and polychaetes (Pernet 1998). Approximately one-third of scaleworms examined by Pernet (1998), however, contained unidentified tissue; he found no indication of the symbiont feeding on its host (e.g. giant red sea cucumber ossicles were not observed in fecal pellets).

Pea crab

At the close of the 19th century, a small crustacean was discovered infesting a large gastropod in southern California, USA, which is the southern extent of the range of *P. californicus* (Lambert 1986). The pinnotherid crab *Opisthopus transversus*, was formally described and named by Rathbun (1894), who went on to provide additional details on the sexual dimorphism of the pea crab and a host list comprising three additional species, including *P. californicus* (Rathbun 1918). Decades later, the relationship between *Parastichopus* and *O. transversus* was confirmed by others (Ricketts and Calvin 1968; Schmitt et al. 1973), the pea crab's range was expanded (Hopkins and Scanland 1964), and the list of host species nearly quadrupled in number (Beondé 1968). From the literature, it can be inferred that: 1) *O. transversus* is the only pinnotherid crab infesting *P. californicus* (Schmitt et al. 1973); 2) once a symbiotic relationship is established, *O. transversus* displays a strong affinity for its host (Ricketts and Calvin 1968); 3) host selection may be linked to host size (e.g. small or young pea crabs infest small hosts, and so on; Hopkins and Scanland 1964); 4) more than one host may be required to complete the life history of *O. transversus*; and 5) different hosts may satisfy different nutrient requirements for the pea crab to develop and reproduce successfully (Beondé 1968; Campos 2016). Alternatively, *O. transversus* may just be experiencing a "Goldilocks moment" in time where, evolutionarily, the species is new to symbiosis and is still determining which host is "just right" (sensu Beondé 1968).

Symbionts and sea cucumber culture: Gleaning new insights by extending the work of others

Surveys of the prevalence of *P. californicus* symbionts in the wild are few. Besides Shinn's (1985) work with the flatworm *A. hymanae*, Cameron (1985) (and later, Cameron and Fankboner 1989) provided useful information on the prevalence of *A. pulchra* and *E. parastichopoli* associated with juvenile and subadult giant red sea cucumber (≤ 4 years old) from BC, Canada; however, the latter's results were based on small sample sizes from a limited number of localities. Recently, Mueller (2016) had the opportunity to collect and inspect a greater number of *P. californicus* of varying sizes and ages, including mature individuals (≥ 5 years old), compared with Canadian researchers. Because his study plan included dissecting every sea cucumber collected for sex determination and maturity, Mueller (2016) was able to check for the prevalence of symbionts across a wide variety of host ages and sizes. The remainder of this paper presents data collected ancillary to a study of the fishery biology of the giant red sea cucumber from the San Juan Islands (Mueller 2016). It is intended to extend the symbiont works of others (e.g. Cameron 1985; Cameron and Fankboner 1989) and to fill in some gaps in our understanding of the ecology of *A. pulchra* and *E. parastichopoli*. A discussion follows on how this information may be of use to those participating in the nascent aquaculture of *P. californicus*.

Materials and methods

Mueller's study (2016) took place in the San Juan Islands where *P. californicus* is abundant relative to other marine areas of Washington State (Bradbury et al. 1998; Carson et al. 2016). The holothurian was collected at several points within four regions (west to east): Upright Channel, Rosario Strait, Bellingham Channel, and the junction of Bellingham, Samish, and Padilla bays (hereafter, "Bays" or Vendovi Island) (Table 2).

Using the same voluntary minimum size limit adopted by many commercial harvest divers as a guideline to retain *P. californicus* (i.e. no shorter than ~5 cm on either side of a gloved hand or ~20 cm whole, contracted length), 50 giant red sea cucumber were collected by divers twice monthly from 7 May 2014 to 15 October 2014, and then again from 17 March 2015 to 28 May 2015. Individual *P. californicus* were placed inside ziplock plastic bags and stored on ice inside a large cooler until being processed the following day at a wet laboratory located on the Northwest Indian College (NWIC) campus in Bellingham. At the NWIC wet laboratory, counts of *A. pulchra* and *E. parastichopoli* were recorded opportunistically for each sea cucumber while other biological data were being gathered (Mueller 2016). The symbionts are relatively large compared with others of the giant red sea cucumber, and are easily identified by an observer without the aid of magnification (Fig. 1 A and B); hence, these qualities simplify determining their presence or absence in the field or laboratory. There was no need to distinguish *A. pulchra* from other species of *Arctonoe* because it is the only scaleworm associated with *P. californicus* in this region of the host's range (Pettibone 1953; Pernet 1999). Furthermore, no indication of size or life stage was recorded for the scaleworm and endoparasitic snail.

By the end of the study, divers had collected 850 *P. californicus* throughout the central-east San Juan

Table 2. Localities where the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) was collected by divers in the San Juan Islands, Washington State, USA during 2014 and 2015. Depth range (m) of the collection refers to that as per diver depth gauge (uncorrected for tidal elevation), whereas average depth (m) of the collection refers to that relative to mean lower low water (MLLW).

Region	Collection point	Latitude	Longitude	Depth range (m, uncorrected)	Average depth (m, MLLW)	No. sea cucumber
Upright Channel	Canoe Island	48°33.561′N	122°55.438′W	6.1 – 15.2	10.7	50
Upright Channel	Canoe Island	48°33.675′N	122°55.433′W	5.5 – 12.2	7.0	50
Upright Channel	Lopez Island	48°33.899′N	122°53.529′W	6.1 – 19.2	11.3	50
Upright Channel	Lopez Island	48°33.913′N	122°53.526′W	6.1 – 24.4	12.8	50
Rosario Strait	Orcas Island	48°36.087′N	122°48.032′W	4.6 – 21.3	11.6	100
Rosario Strait	Blakely Island	48°35.028′N	122°47.930′W	12.2 – 20.4	14.0	50
Rosario Strait	Cypress Island	48°35.867′N	122°43.885′W	33.5 – 36.6	33.5	50
Rosario Strait	Cypress Island	48°36.184′N	122°43.542′W	4.9 – 12.2	9.8	50
Bellingham Channel	Cone Islands	48°35.564′N	122°41.021′W	6.1 – 12.2	9.1	77
Bellingham Channel	Cone Islands	48°35.520′N	122°40.579′W	3.4 – 15.2	8.5	100
Bellingham Channel	Sinclair Island	48°36.642′N	122°40.768′W	5.5 – 13.7	5.2	50
Bellingham Channel	Sinclair Island	48°36.458′N	122°39.983′W	6.1 – 12.2	9.1	23
"Bays"	Vendovi Island	48°36.821′N	122°36.886′W	6.1 – 21.3	10.4	150

Islands at average depths ranging from 5.2 m to 33.5 m (mean lower low water) (Table 2). Frequency distributions of *A. pulchra* and *E. parastichopoli* were calculated for host collection date and locality, host sex, and age and size of the giant red sea cucumber. Symbiont prevalence was determined after Bush et al. (1997) as the number of hosts infected or infested with one or more individuals of a symbiont species divided by the number of hosts examined for that species. Proportional prevalence of the symbionts were then plotted at three levels (0, 1, and ≥ 2 organisms per sea cucumber) for each of the factors except host sex. Symbiont intensity was also determined after Bush et al. (1997) as the number of individuals of a symbiont species in a single infected or infested host. Age and size of *P. californicus* were determined using methods described by Mueller (2016).

Results

The commensal scaleworm *A. pulchra* infested up to 70% of *P. californicus* collected in 2014 and 2015. The intensity of an infestation was no more than three scaleworms per sea cucumber. Proportional prevalence of *A. pulchra* was ostensibly influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, proportional prevalence of *A. pulchra* increased during the spring of both study years, peaking in mid-summer 2014 and again in mid-fall 2014 (Fig. 2A). Between host collection localities, the proportional prevalence of *A. pulchra* varied by as much as 0.2. While no clear longitudinal trend was observed in sea cucumbers with one scaleworm attached, there was a slight increase in the proportional prevalence of *A. pulchra* at higher densities (i.e. ≥ 2 polychaetes per sea cucumber) moving from west to east in the San Juan Islands (Fig. 3A). In addition, the proportional prevalence of *A. pulchra* increased with the age and size of *P. californicus* collected (Fig. 4A); however, the sex of *P. californicus* did not appear to greatly influence the proportional prevalence of the scaleworm. Indeed, 179 of 364 female *P. californicus* were infested by at least one *A. pulchra* (proportional prevalence = 0.49), whereas 150 of 369 male *P. californicus* were infested by at least one scaleworm (proportional prevalence = 0.41). Lastly, at least one *A. pulchra* infested 60 of 117 giant red sea cucumbers of unknown sex (proportional prevalence = 0.51).

The shell-less, endoparasitic snail *E. parastichopoli*, on the other hand, infected up to 30% of *P. californicus* collected in 2014 and 2015. The intensity of *E. parastichopoli* infections were variable; usually, there were no more than one or two endoparasitic snails per sea cucumber, but intensities as high as 42 per sea cucumber were observed (Fig. 1B). Both juvenile and adult stages of the symbiont comprised the highest intensities of infection, whereas lower intensities were characterized by the presence of adult stages only. Like *A. pulchra*, the proportional prevalence of *E. parastichopoli* appeared to be influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, the proportional presence of *E. parastichopoli* increased slightly during spring, but decreased mid-summer 2014, and decreased further by the last sampling date in fall 2014 (Fig. 2B). In terms of host collection locality (Table 2), the highest proportional

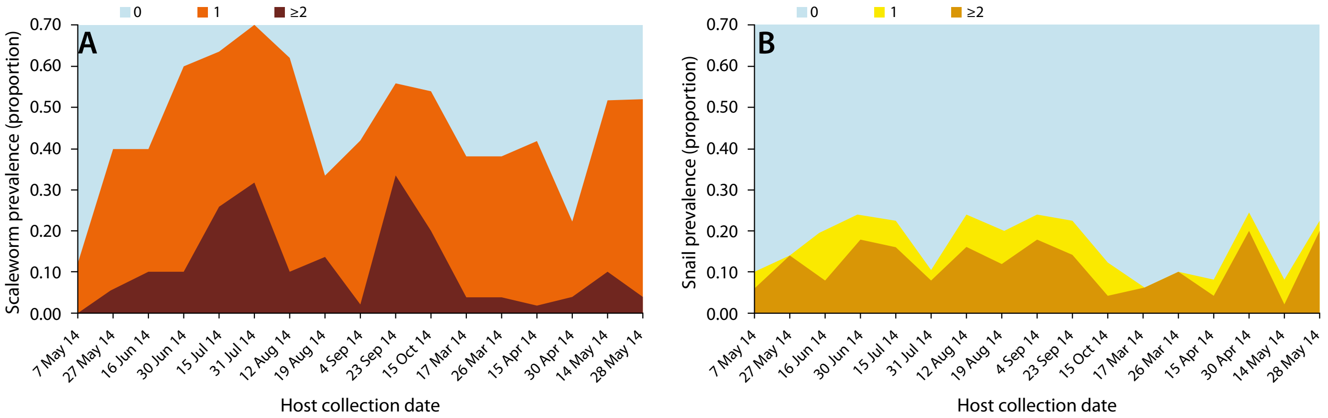

Figure 2. Proportional prevalence of symbionts of the giant red sea cucumber *Parastichopus californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection date. Fifty giant red sea cucumbers were collected on each date. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

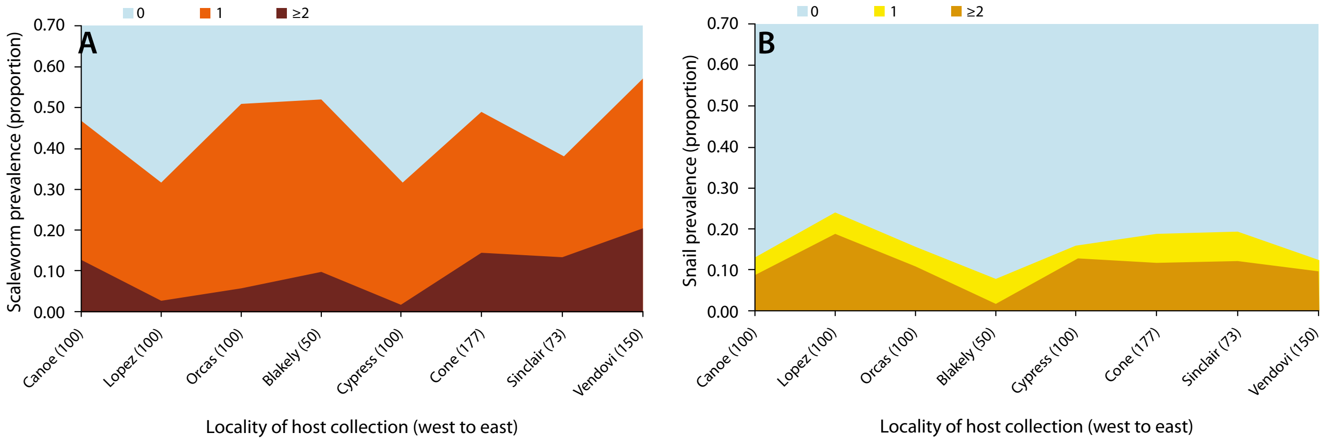

Figure 3. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection locality (ordered west to east). The number of giant red sea cucumbers collected by locality is indicated parenthetically. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

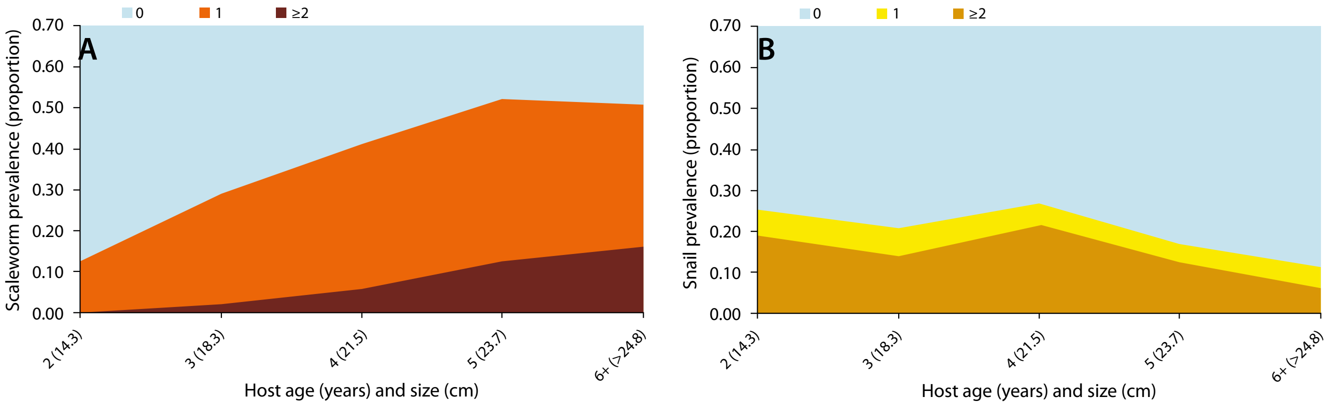

Figure 4. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host age (years) and size (cm, indicated parenthetically). The numbers of giant red sea cucumber collected for each age and size group were as follows: age 2, *n* = 16; age 3, *n* = 107; age 4, *n* = 158; age 5, *n* = 161; and age 6+, *n* = 408. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

prevalence of endoparasitic snails occurred at Lopez Island (Upright Channel) and in the vicinity of Sinclair and the Cone islands (Bellingham Channel; Fig. 3B). And while the proportional prevalence of *E. parastichopoli* in immature *P. californicus* (age = 2–4 years) fluctuated between 0.20 and 0.25, the proportional prevalence of endoparasitic snails in mature sea cucumbers (age ≥ 5 years) fell below 0.15 (Fig. 4B). Regarding sex of the host, 50 out of 364 female *P. californicus* were infected with at least one endoparasitic snail (proportional prevalence = 0.14), whereas 56 of 369 male *P. californicus* were infected with at least one *E. parastichopoli* (proportional prevalence = 0.15). In terms of giant red sea cucumbers of unknown sex, 34 out of 117 were infected with at least one endoparasitic snail (proportional prevalence = 0.29).

Ultimately, irrespective of host collection date and locality, and age and size of *P. californicus*, there appeared to be an inverse relationship between the proportional prevalence of the commensal scaleworm and the proportional prevalence of the endoparasitic snail: whenever or wherever there was an increase in the proportional prevalence of *A. pulchra*, there was a subsequent decrease in t hepproportional prevalence of *E. parastichopoli* and vice versa (Figs. 2–4).

Discussion

At present, the symbiont community of the giant red sea cucumber (Table 1) does not appear to affect its host too profoundly in the wild (Stevens 1901; Tikasingh 1962; Fankboner and Cameron 1985; Pernet 1998; Bower 2004a, b); however, when placed into an aquaculture setting, one or more of the naturally occurring symbionts of *P. californicus* could become pathogenic or become a pathway or vector for disease (Blaylock and Bullard 2014; Shinn et al. 2015; Overstreet and Lotz 2016). For example, Wang et al. (2004) reported high intensities of a protozoan ciliate inhabiting the respiratory tree of cultured *Apostichopus japonicus* (Selenka, 1867) [likely *Boveria labialis* (Ikeda and Ozaki, 1918)] that weakened the host and occasionally led to the ejection of the sea cucumber's coelomic contents. In fact, Long et al. (2006) reported secondary bacteriosis in *A. japonicus* as a result of *B. labialis* infections. Furthermore, crustaceans (Brachyura, Copepoda, and Isopoda) living in sympatry with cultured *A. japonicus* and *Holothuria scabra* (Jaeger, 1833) were identified as potential sources of high mortality in the sea cucumbers (Wang et al. 2004; Lavitra et al. 2009). Moving forward, culturists of *P. californicus* should be particularly aware of the scuticociliate *B. subcylindrica*, and in the southern extent of the host's range, the pea crab *O. transversus*. Although the latter was described as leaving its host periodically to forage (Beondé 1968), the dynamics between host and symbiont could change in an intensive culture setting.

Prior to this study, data on the natural prevalence of *A. pulchra* were limited. For example, Cameron (1985) (and later, Cameron and Fankboner 1989) collected and inspected between 11 and 42 juvenile *P. californicus* (age ≤ 1 year; whole, contracted length < 2 cm) from each of 14 samples taken across four localities in BC, Canada. The author(s) did not report the total number of juvenile giant red sea cucumbers collected, nor did they report the number collected by locality. It can be assumed, however, that the *minimum* total number of juvenile giant red sea cucumbers collected by them was ≥ 154 (i.e. 11 sea cucumbers X 14 samples). Of all these, only 3 of the 42 juvenile giant red sea cucumbers collected during one trip to one locality were infested with a single, small *A. pulchra* (proportional prevalence = 0.071). Extending this exercise to the regional scale (i.e. all four localities combined), the proportional prevalence of *A. pulchra* on juvenile *P. californicus* can be estimated as the number of infested giant red sea cucumbers (n = 3) divided by the total number of holothurians from all 14 samples (n range = 11–42 *P. californicus* per sample); or, put another way, the estimate of regional proportional prevalence of the scaleworm on juvenile giant red sea cucumbers during Cameron's (1985) study was between 0.005 [= 3/(42 X 14)] and 0.019 [= 3/(11 X 14)]. In contrast, Pernet (1998) noted a 10-fold increase (or more) in the proportional prevalence of *A. pulchra* (= 0.20) infesting adult-size *P. californicus* from the San Juan Islands, yet both of these examples fall short of the summertime peak (proportional prevalence = 0.70) reported in the present study. Such spatiotemporal differences in the prevalence of symbionts have been widely reported throughout the animal kingdom (e.g. Martin and Britayev 1998; Timi and Poulin 2003; Hoffmann et al. 2016).

Data on the natural prevalence of *E. parastichopoli*, while also limited, have at least been better documented than those for the scaleworm. For example, Harrington and Griffin (1897) reported that 3 of the 30 or 40 *P. californicus* that were examined were infected by endoparasitic snails (proportional prevalence = 0.07–0.10), while Tikasingh (1960) found that just 14 out of 276 giant red sea cucumbers examined in mid-summer to early fall were infected by *E. parastichopoli* (proportional prevalence = 0.05). Furthermore, Jangoux (1987b), citing Lützen's (1979) findings, tabulated intensities of approximately three *E. parastichopoli* per host in 37 out of 244 *P. californicus* examined (proportional prevalence = 0.15), whereas Cameron and Fankboner (1989) reported a proportional prevalence of 0.09 during August 1983 (6 out of 63 subadult *P. californicus* aged 2–4 years) inspected were infected by *E. parastichopoli*, and no endoparasitic snails were found in 36 giant red sea cucumbers examined in October 1983 at the onset of aestivation following the visceral atrophy phase. All of these findings are consistent with those from various collection dates and localities and host ages and sizes reported in the present study.

The inverse relationship between *A. pulchra* and *E. parastichopoli* is particularly intriguing. This is the first study to provide evidence of a possible interaction between the two symbionts. Early research on the endoparasitic snail suggested that the symbiont entered *P. californicus* either orally or anally, but mostly while the host fed on the earliest life stages of *E. parastichopoli* (Tikasingh 1960, 1961, 1962; Kincaid 1964). Hypotheses regarding other modes of host–symbiont contact between the giant red sea cucumber and the endoparasitic snail were never made. Only in the past decade was an alternative documented: Altnöder et al. (2007) provided photographic evidence of microscopic, shelled larvae of an endoparasitic snail attached to the skin of its holothurian host. Regarding the possible interaction between *A. pulchra* and *E. parastichopoli*, it is proposed that the scaleworm preys on the shelled larvae of the endoparasitic snail when the former encounters the latter attached to the skin of *P. californicus*. The two symbionts might also encounter each other in the buccal area of the host where *A. pulchra* is often found (Pettibone 1953). Indeed, the scaleworm readily feeds on larval bivalves and gastropods (Mollusca) in a laboratory setting (Pernet 2000); hence, predator-prey dynamics might contribute to the observed inverse relationship between the two symbionts of *P. californicus*.

Interspecific interactions among symbionts have been investigated in both laboratory and natural settings (Pugachev 2000; Timi and Poulin 2003; Fenton et al. 2014; Hoffmann et al. 2016). One example of an obvious interaction between symbionts of *P. californicus* is Stevens' (1901) record of the ciliate *L. macfarlandi* preying directly upon the smaller ciliate *B. subcylindrica* within the respiratory tree of the host. Conversely, Timi and Poulin (2003) found that changes in a host's symbiont community structure were due mostly to abiotic factors rather than interactions between symbionts. Fenton et al. (2014) cautioned that several underlying factors at the logical, temporal, and spatial scale might contribute to observed variation in symbiont prevalence and intensity that would otherwise be attributed to interspecific interactions. These authors suggested experimental and statistical approaches that would help separate interspecific interactions from other factors, potentially improving their detection. Additional causes for the observed relationship between *A. pulchra* and *E. parastichopoli* may, therefore, be revealed by applying more rigorous analytical methods *sensu* Fenton et al. (2014).

Until such work is completed, though, it is recommended that the commensal relationship between the giant red sea cucumber and *A. pulchra* be maintained in future aquaculture settings. In this way, predation by *A. pulchra* might serve as a natural prophylactic treatment for parasitosis, thereby reducing the risk of high intensity infections of *E. parastichopoli*. Laboratory cultures of the scaleworm (Pernet 1998, 1999, 2000) could even be maintained for this purpose. Given the evidence presented here for the probable interaction between the two symbionts at multiple scales (time, space and host), this simple measure should be a prudent element of any health management plan for *P. californicus* culture. Natural treatment of a pest via predation in sea cucumber aquaculture is not without precedent. For example, Lavitra et al. (2009) described intentional introductions of a carnivorous fish into commercial sea cucumber ponds to successfully eliminate a deleterious, pathogenic crustacean (Isopoda). And should the ciliate *B. subcylindrica* ever become as problematic to giant red sea cucumber culture as its congener, *B. labialis* is to *Actinopyga japonicus* culture, might predation by the larger ciliate *L. macfarlandi* be a possible solution?

To summarize, this paper provides the essential first step of reviewing known symbionts of the giant red sea cucumber and delves into the ecology of two of them on multiple levels (time, space and host), providing some possible directions for future research. For example, does the higher proportional prevalence of *E. parastichopoli* infecting *P. californicus* of unknown sex (0.29) vs those of male and female giant red sea cucumbers (0.15 and 0.14, respectively) indicate a negative impact on reproductive capacity of the host? Current knowledge of the relationship between endoparasitic gastropods and their echinoderm hosts suggests this may be unlikely (Jangoux 1987b); still, it is difficult to imagine that a moderate to severe infection of *E. parastichopoli* (Fig. 1B) has no ecological consequences for *P. californicus*. And what drives higher the proportional prevalence of *A. pulchra* in mature *P. californicus* (≥ 5 years old) compared with younger giant red sea cucumbers? The host's body size? The host's reproductive status?

In conclusion, the diversity of symbionts associated with the giant red sea cucumber will challenge future *P. californicus* culturists and hatchery workers with predicting the effects of disease or pest outbreaks and subsequent treatment protocols (Fenton et al. 2014; Shinn et al. 2015). While knowing the symbiont composition in *P. californicus* (Table 1) is an essential step in "biosecurity" (i.e. minimizing the risk of introducing or transmitting disease or pest agents) of the host species (Yanong and Erlacher-Reid 2012), other lessons to be learned include improved understanding of how symbiont diversity affects infections or infestations, how sea cucumber culture affects genetic diversity of *P. californicus* (and subsequently, its relationships with symbionts), natural patterns in symbiont communities across different scales, and symbiont interactions at the host level (Johnson et al. 2015). This information can be used by culturists and hatchery workers when establishing health management plans for, and identifying possible triggers of, harmful conditions affecting reared *P. californicus* (Wang et al. 2004; Blaylock and Bullard 2014; Overstreet and Lotz 2016).

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Revision of the geographical range of *Actinopyga capillata* Rowe & Massin 2006 (Echinodermata: Holothuroidea)

Frédéric Ducarme[1]

Abstract

This paper updates the geographical range of the holothurian species *Actinopyga capillata* (Rowe & Massin 2006), first described as endemic from the Mascarene Islands with one ectopic record from Philippines. New observations confirm the presence of this species in different regions of the Philippines, thereby extending its range westward to Madagascar and the Comoro Islands, suggesting a wide but discontinuous Indo-Pacific range. Neither fishing nor trade of this species has yet been recorded.

Introduction

Sea cucumbers (Holothuroidea) constitute a group of marine invertebrates experiencing important and still poorly regulated fisheries and an international trade that is, essentially, aimed at Southeast Asian markets (Eriksson et al. 2015; Conand, in press). Therefore, knowledge on their distribution and population dynamics is paramount, as many populations have already been jeopardised even before sound scientific knowledge was made available (James and Manikfan 1994). *Actinopyga capillata* (Rowe & Massin 2006) is the most recently described species of one genus of commercially important sea cucumbers, hence a potential target for trade and conservation; 10 years after its description, we propose here an update on its geographical range.

Geographical re-assessment

The "hairy sea cucumber" *Actinopyga capillata* was described in 2006 (Rowe & Massin 2006) from specimens collected in La Réunion (holotype) and Rodrigues (paratypes), both islands of the Mascarenes archipelago in the southwest Indian Ocean. The original publication also mentioned visual observations from Mauritius (third island of the Mascarenes) and, farther away, the Philippines. No other observations have been recorded since then, according to the International Union for Conservation of Nature (IUCN) (Conand et al. 2013). Visual observations were considered safe by authors as this species displays very characteristic morphological traits preventing any ambiguity: "the shape of the body, together with the distinctive arrangement of the elongate, modified dorsal tube feet, and the ossicle complement, all set this species apart from others in the genus *Actinopyga*. The live colour pattern is also distinctive for *A. capillata* n. sp., within the genus" (Rowe and Massin 2006), the genus being already quite readily identified thanks to its distinctive anal teeth (Samyn et al. 2006). These traits allow the confirmation of new records of this species based on visual observations or good photographs without sampling, yet keeping in mind that in the absence of DNA analysis the west Pacific, specimens might still belong to a morphologically identical sister taxa, which can happen in echinoderms.

Field investigations at Mayotte Island (Comoros archipelago) allowed the observation of several unambiguous specimens of *A. capillata*, recording its presence in the area on shallow inner reefs at night (Fig. 1). This constitutes a first record in the region, as this species is absent from previous local inventories such as Eriksson et al. (2012). We consider that the absence of this species from previous inventories is more likely due to sampling methods or natural abundance variations.

Photographs taken in Madagascar by Fabrice Rozier, and confirmed by experts, suggest the presence of this species in the region of Toliara, southwest of Madagascar, perhaps with a gene flow between the neighbouring archipelagos of the Comoros and Mascarenes.

Photographs taken in the Philippines by Oliver Robillo, and uploaded onto the *iNaturalist* website and confirmed by experts, confirm the presence of *Actinopyga capillata* at Talikud Island, south of the Philippines (Davao region). Another photograph was taken by Sylvain Le Bris at Siquijor Island, central Philippines (Central Visayas region), and posted on the French diving forum DORIS, as an answer to a call for observations. No other photograph could be found on naturalist websites from surrounding regions such as Indonesia, Thailand, Australia, New Caledonia or the Pacific Islands.

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Figure 1. Specimen of *Actinopyga capillata* observed in Mayotte (Sakouli inner reef, 1 m depth at night). (Image: F. Ducarme).

Recent sea cucumbers inventories from the Indian Ocean (e.g. Ducarme 2016 in the Maldives; Conand et al. 2015 in the Scattered Islands) did not record any specimen of *A. capillata*, suggesting either a particularly cryptic behaviour in some countries, or a discontinuous geographical range. The nocturnal behaviour of *Actinopyga capillata* (already documented by Rowe & Massin 2006 and confirmed here) could also prevent this species from being observed, as well as its modest size compared with other species of the same genus (rarely more than 15 cm, compared with over 40 cm for many other *Actinopyga*). Scientists and marine naturalists from surrounding regions contacted in the frame of this work did not mention any observation in their field works. To date, no observation has been reported from African coasts, but the species might have some populations there, in continuity with the Comoros and Madagascar, as happens for many sea cucumber species in the region (there is no significantly more important biological barrier between Comoros and east Africa than between Madagascar and the Mascarenes).

Discussion

The current confirmed geographical range of *Actinopyga capillata* extends from Mayotte to Mauritius, with a separate population in the Philippines; however, we can hypothesise a theoretical range extending from east Africa to the western Pacific Ocean, with potential discontinuity.

Although it is a recently described species, *Actinopyga capillata* may be already known by local fishermen in many countries. As part of the high commercial value genus *Actinopyga*, it may also constitute a potential target for the beche-de-mer fishery and trade, even if it is not currently cited among the commercially important sea cucumbers of the world (Purcell et al. 2012). This species does not seem to be present on Asian markets so far (C. Conand, pers. comm.), perhaps because it is a rather small species and is rarely abundant in its range, and often observed isolated (despite reported higher densities in Mauritius according to Rowe & Massin 2006). This species is still considered to be "data deficient" by the IUCN (Conand et al. 2013), and more investigations are needed to assess its population dynamics; however, this range extension and the absence of evidence for fishing or trade suggest that this species may not be threatened in the short term. Little is known about its ecology, and the new observations gathered here tend to confirm the original description (i.e. inhabiting shallow areas and being nocturnal).

Acknowledgements

I am thankful to the three underwater photographers who provided pictures of the species, Blogie Robillo, Fabrice Rozier and Sylvain Le Bris. I also thank Chantal Conand, Philippe Bourjon and Yves Samyn for their help and advice, and Rémy Eudeline for his field support.

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Development of techniques for gender identification in *Holothuria forskali* (Delle Chiaje, 1823)

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Abstract

Holothuria forskali is a widely distributed sea cucumber species in the Atlantic Ocean and Mediterranean. Understanding the reproductive biology study of *H. forskali* is a crucial step towards achieving the sustainable production of this species in aquaculture facilities. Because echinoderms have no sexual dimorphism, it is not possible to determine their gender externally. This study aims to apply four different techniques for determining the gender of holothurians through the collection of a piece of gonad: biopsy, aspiration without incision, a short incision in the dorsal side, and a short cut in the anterior part. The biopsy method showed the highest percentage of accuracy, with 100% of gender identification compared with the other methods. In the two methods with incision, specimens were fully recovered after 21 days, showing no signs of scars or any evidence of the cut made.

Introduction

Echinoderms show no sexual dimorphism, so externally it is not possible to distinguish between males and females (Yahyavi et al. 2012). To improve broodstock conditioning of *Holothuria forskali*, gender identification is an important step. The existing methods of gender identification were only reported in studies of rearing, but with only a few descriptions (Battaglene 1999; Morgan 2000). According to Santos et al. (2015), the tubule shape of the gonads of *Holothuria forskali* is the first way to evaluate gender. When they are mature, it is possible to see (macroscopically) the oocytes inside the tubule walls, and the lumen filled by mature spermatocytes. The gonads of mature females have a distinct orange colour and the males, a strong salmon-pink colour (Keshavarz et al. 2012; Ramofafia et al. 2000). This study aimed to determine gender without causing evisceration or consequential mortality, through the application of four different methods: aspiration of a piece of gonad using a biopsy needle, aspiration of the gonad blunt, cutting the dorsal part with suction, and cutting the anterior part. The regeneration ability of this species was evaluated.

Methods

Sampling

Holothuria forskali were captured in Quebrado beach Peniche (39°22′3″N 9°22′26″ W) (Fig. 1), during low tide on the Peniche coast (39°21′14.4 ″N and

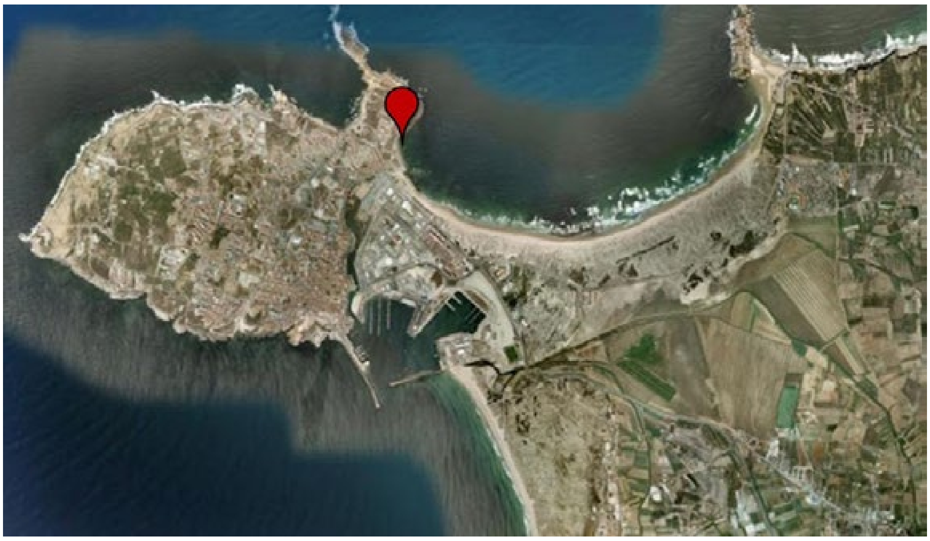

Figure 1. Peniche, Portugal. The defined area corresponding to the geographical area where *Holothuria forskali* were collected.

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9°23′43.7″ W), to obtain a total of 80 organisms. Sea cucumbers were caught by hand and transported in 4 groups of 20 individuals to 20-L plastic containers. Individuals were brought immediately to the Aquaculture Lab of MARE (Marine and Environmental Sciences Centre)-IPLeiria, where they were kept in 60-L tanks with recirculation systems. The methods were applied after a seven-day period of adaptation, to reduce physiological stress.

Conditioning

For each method, 20 individuals were conditioned in two 60-L tanks (10 sea cucumbers in each) in a recirculation system. The first tank was used as a control in which the animals were not subject to any method, and the second tank was the experimental tank. The temperature was regulated by a refrigeration system (FRIMAR 300, Portugal), maintained at 16°C. Holothurians were fed with a mixture of four microalgae species: *Tetraselmis suecica*, *Clorella sp.*, *Phaeodactylum tricornutum* and *Isochrysis galbana* (Ivy and Giraspy 2006), provided once a day, in the morning, in the same proportion, according to the tank volume in a concentration approximately of 1.5×10^5 cells mL^{-1} throughout the entire experimental period (Battaglene et al. 2002). Water quality parameters such as dissolved oxygen and temperature (INNOVAQUA, Spain), ammonia and nitrites were measured weekly (Hanna Instrument Ammonia and Nitrite Test Kit for Salt Water).

Method 1 — Biopsy method

The first method was based on studies made by Battaglene (1999) and Morgan (2000). The sex ratio was found using a biopsy needle (Fig. 2) to collect some gametes that were observed and photographed with a Leica DM microscope (Leica, Bensheim, Germany).

Figure 2. Method 1 — A biopsy needle used for gonad sampling of *Holothuria forskali*.

Method 2 — Aspiration without incision

This method was based on experiments performed by Battaglene (1999) and Al-Rashdi et al. (2007). Each animal was picked up and squeezed on the ventral side in order to push all organs to the anterior end. Afterwards, a Pasteur pipette was placed into the mouth and an attempt was made to pull part out of the gonads, making it possible to identify the gender of the individual. In some animals, it was possible to observe the shape and colour of the gonad and macroscopically identify the respective gender. In individuals where sexual identification was in question, a microscopic (Leica DM (Leica, Bensheim, Germany) observation of the gametes was made.

Method 3 — Incision in dorsal side

The third method emerged as an adaptation of the work described by Menton and Eisen (1973) and Yanagisawa (1998). This method was based on making a small incision in the dorsal area of the body (Fig. 3). The body was placed on a tray by making a small cut in the tegument with a scalpel. Once the section was made, a Pasteur pipette was placed in the opening and part of the gonad was pulled on.

Figure 3. *Holothuria forskali* after a cut was made on its dorsal side (Method 3).

Method 4 — Cut in anterior part

This method is based on previous works (Battaglene 1999; Menton and Eisen 1973; Yanagisawa 1998). It consisted of making a small cut at the forward end of the body. Using scissors, a small incision was made in the mouthpiece, providing a larger opening for collecting part of the gonad. After cutting, a Pasteur pipette was inserted to aspirate part of the gonads, which were then collected by tweezers and a microscopic examination of them was performed.

After the application of each method, individuals were left to recover for 21 days, without manipulation, in recirculation aquaculture systems, although food supply was maintained. Mortality and/or evisceration were recorded daily. In cases of evisceration, the gonads were collected to determine the maturity stage (Keshavarz et al. 2012; Ramofafia et al. 2003).

Results

During the trial and recovery period, temperature was maintained at 16 ± 1°C, and salinity and dissolved oxygen were maintained at 33 ± 1 ppt and 8.0 ± 0.2 mg $L^{-1,}$ respectively, in all tanks. Neither mortality nor evisceration were recorded using the first, third and fourth methods. It was observed that with the second method, aspiration without incision resulted in evisceration and mortality. The survival rate obtained for the second method was 80% and was 100% in the control tank.

Gender determination

The results of gender determination for all four methods are shown in Figure 4. A success rate of 100% was achieved using the first method, 40% for the second and third methods each, and 70% for the fourth method. For the third and fourth methods, specimens showed a full recovery 21 days after the method was conducted, showing no signs of the incision that was made.

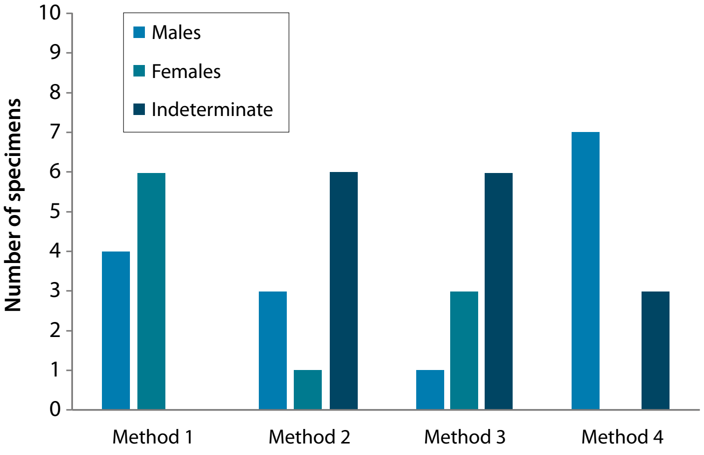

Figure 4. Total number of males, females or indeterminate individuals in each performed method.

Mortality and evisceration that occurred in the second method are associated with handling procedures, more specifically, when the animals were squeezed in the ventral side to push all organs to the anterior end. This can be confirmed by 30% evisceration and 20% mortality using this method. Despite these results, the identification had the same success as the third method, in which there was no mortality or evisceration. In the third method where there is an incision in the dorsal side, the organs were exposed through the cut made, but it was not always possible to see the gonads, and therefore better results were obtained using the first method.

Regarding the fourth method, the cut made in the anterior area caused relaxation of the mouth muscles, which made it was possible to insert the tweezers. In this method it was not possible to see the gonads at first, but through compression, it was possible to locate and extract the gonadal tubules. With this method, it was possible to remove a tubule of each individual gonad, thus identifying the gender. This method differs from the second method because it was made by a cut, using tweezers to grab the gonads instead of a Pasteur pipette. This fact may explain the easiness in obtaining the entire gonad tubules.

In all methods, the aspirated content had perceptible oocytes in the case of females, and a lumen filled with mature spermatocytes, in males. There are surprisingly few studies based on skin healing in holothurians (Yanagisawa 1998). Despite the evasive methods that were performed where a cut was applied, *Holothuria forskali* has developed a mechanism capable of recovering from a cut. The described time for complete regeneration of a wound was three to four weeks, including for an organ or an appendage, and the sea cucumber is able to regenerate a missing structure (Cowden 1968; Menton and Eisen 1973; Sun et al. 2011). In the present study, it was observed that specimens had no scar or other traces that indicated a cut had been made within the recovery time of 21 days. In conclusion, getting information on the gender identification can made a successful broodstock collection, thus enhancing the success of breeding programmes with new sea cucumber species.

Discussion and conclusion

According to Morgan (2000), the extraction of gonadal material is useful in determining the sex of sea cucumbers. Once this technique had been refined, no animals eviscerated after a puncture of the viscera with the biopsy needle. In our study, with the first method, 100% of gender identification was made without any detection of mortality or evisceration.

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Teripang fishing activities at Barang Lompo Island, Sulawesi, Indonesia: An update 20 years after a visit in 1996

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Abstract

The current situation regarding *teripang* (dried sea cucumber) activities at Barang Lompo Island is presented in this article, and is based on interviews and visits to processors. The comparison with a visit 20 years ago shows that this activity is still important as fishermen continue to go for long trips far from the island to collect *teripang*, but there have been noticeable changes in the depth of the dives, the species collected, the catch volume and processing methods. The present high prices paid for processed product compensates fishermen for the decrease in capture volume.

Introduction

Indonesia has been and still remains the top producer of dried sea cucumber (*teripang*) on a global scale (Conand 1990; Choo 2008; Conand in press). The *teripang* fishery and trade have already been described from several islands in Indonesia (e.g. Tuwo and Conand 1992; Moore 1998; Navarro et al. 2014; Setyastuti and Purwati 2015). Barang Lompo is a small island in the Spermonde Archipelago near Makassar, South Sulawesi where this fishery has traditionally been important. Following a first visit in 1996 (Conand and Tuwo 1996), the current state of activities was assessed during a visit in 2016, where the authors were able to hold interviews and make observations.

Material and methods

Interviews were conducted with a *teripang* diver at the University Hasanuddin's Marine Station and at two different *teripang* processing centres on Barong Lompo Island. Samples of several species were observed, collected and measured.

The results are discussed in comparison with observations made during a visit to Barang Lompo 20 years ago. The Food and Agriculture Organization guide (Purcell et al. 2012) was useful for identification, although more species need to be included to allow for changes in target species.

Figure 1. Barang Lompo Island with some fishing boats moored around the island. (Google maps: https://www.google.co.id/maps/).

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Results

Interview with a teripang fisherman

Fishing trips and crew

"PK" is 41 years old. He has spent 25 years diving for sea cucumbers and arrived in Barang Lompo from another island 10 years ago because he found a "boss" here. His fishing grounds include different sites in the Sulawesi Sea, Java Sea, the coasts of south and west Borneo, Makassar Strait, and the Mollucas to the Australian offshore reefs of Cartier Island (called Pasir Putih by Indonesians). He usually goes on fishing trips that last about a month, and generally makes seven trips per year, with 5–10-day breaks to rest between trips.

The boat is owned by a Barang Lompo resident, and the official registered tonnage is around 7 gross tons. The crew of 10 comprises 6 divers, the captain, the compressor operators, and the engineer. Bottled gas is used on the boat for cooking and for *teripang* processing. There are several dozen vessels that go to the same sites, some of similar size and others slightly smaller with eight crew members, five of whom are divers.

The crew, including the divers, may switch between boats; for example, PK has worked on 20 different boats successively. The provisions loaded on the boat are calculated to enable the crew to remain at sea for up to 40 days. Provisions include rice and fuel, as well as the cigarettes (around 200 multipack boxes), which represent an important part of the crew's remuneration. There is a complex system for the division of profits, with the divers only receiving a small percentage.

If a diver suffers a decompression accident, he will generally go back down with another diver who will give him an underwater massage, before they both return very slowly to the surface. The family receives a one-off payment in case of death but there is no such compensation in the event of permanent disability.

Hookah diving

Diving activities last for around eight hours each day (from 08:00 to 16:00). The three pairs of divers take turns going down, with each pair collecting for 30 minutes then resting for an hour. They dive three times per day using hookah gear, mostly to depths of 20–30 m (sometimes deeper, to 40 m or more). The twin hoses are 140 m in length and fitted with regulators. The divers are aware when they go deeper than 20 m because the regulator makes a special noise below when the diver goes below this point. The boat has been equipped with an echosounder for five years, so the crew can "see" the bottom depth and profile; previously, they used a weighted rope to determine the depth. The divers pick the sea cucumbers up by hand and place them in a mesh bag.

Species targeted and processing onboard

The main large species currently being collected include *teripang biba* (probably *Holothuria lessoni*) referred to as the "big *taikongkong*" (TKK). Current onboard processing techniques comprise several steps. Sea cucumbers are first "sanded" until they are white in order to remove the surface spicules, then cooked for 15 minutes in sea water. They are then cut open on the ventral side for evisceration and cleaning; they are then salted (with salt pushed into the body cavity and packed between individuals to prevent contact between them) and stored in a polystyrene box for 24 hours. The next day, they are cooked again, drained and put in a drum with salt where they can be stored for several months.

The other species, *teripang gondrong* (meaning long-haired sea cucumber, probably *Stichopus naso*), is much smaller. These are not sanded but are eviscerated, cleaned and cooked onboard. On return to

Figure 2. Processed *teripang* at the first processor. A: *teripang biba*, *Holothuria lessoni*; B: several well-processed species. (images: C. Conand)

Barang Lompo, the buyer cleans the semi-processed *teripang* once more, and proceeds to the final cooking stage just before they are taken to Makassar.

Sampling at the first processor: The (recently arrived) catch observed consisted mostly of *teripang biba* (probably *Holothuria lessoni*), which were around 30 cm in length after the final (third) cooking. Other species observed were *Stichopus hermani*, or big *taikongkong besar*, where were around 17 cm in length, *Thelenota ananas* and *H. whitmaei* (Fig. 2). Processing is done by the brother-in-law of the owner who has 20 boats. Products from this processor are mostly sent to Hong Kong.

Sampling at the second processor: Many TKK *gondrong* (probably *Stichopus naso*) were observed. This species has been described in detail by Massin (2007). It shrinks significantly during processing and is soaked in fresh water giving it the name of *teripang rendam* (soaked *teripang*). It used to have very little value but is now in high demand. In a batch recently landed from a teripang fishing boat, the mean observed length was 12 cm.

At this processor, many different species were observed in baskets that had not been sorted by species. Species included: teatfish *Holothuria whitmaei* (few specimens from 17 to 20 cm), *Thelenota ananas* (few specimens 21 to 25 cm), *H. edulis* (15 to 19 cm), *H. fuscopunctata*, *H. scabra*, and finally the dendrochirotid *Pseudocholochirus* sp. (around 9 cm), locally called *teripang kurma* (meaning date *teripang*), which was never observed before as a processed product (see other communication in this issue).

Discussion

Sea cucumbers represent the most important target species for fishing boats of Barang Lompo Island. Any additional catch is considered to be the individual property of the collecting diver, thereby providing non-predictable but welcome additional income. Typically, this might include lobsters (*Palinurus* sp., *lobster bamboo* and *mutiara*) which are kept alive long enough to be sold, usually before making the return journey; some gastropods, including trochus, *Charonia tritonis* (very rare) and *Cassus cornutus* (*kepala kambing*), all three of which are protected species; and the bivalve *Pinctada margaritifera*.

There are presently 4 main processors, 30 boat owners (1 to 5 boats each), and 20 traders taking product to Makassar. In a typical trip, one boat may catch up to 2 t of wet sea cucumbers. In 1996, boats only carried compasses to find the fishing grounds, but now most use a GPS.

The comparison with the situation observed in 1996 (Conand and Tuwo 1996) is based on an interview with the diver in 2016 who noted the following:

> The fishing grounds today are about the same distance from Barang Lompo as they were before, but are not in the same area. Now, many fishers come from different places (e.g. Madura, Java, Sulawesi, West Nusa Tenggara and others) and venture out the same distance from Barang Lompo as before but to different sites, so there is no conflict among the fishers.
>
> There have been changes in the fishery over the last 20 years, including a decline in sea cucumber catches but an increase in prices, where the income earned is roughly the same as it was 20 years ago.
>
> Divers still go down to about the same depth as before, but are spending more time in deeper water.
>
> Divers used to dive in Australian waters (near Cartier Island), but now fish just outside of this area.

From our previous observations, it appears that the overall sea cucumber fishery on the island has declined since 1996.

> We observed 30 fishermen on 30 boats who did not process the catch onboard but instead, kept the catch in salt. The

Figure 3. A: The last step of cooking TKK *gondrong*; B: TKK *gondrong Stichopus naso* (?); C: several species, including the orange "date *teripang*", *Pseudocholochirus* sp. (images: C. Conand)

divers were mostly Barang Lompo inhabitants but recently, they have been coming from other islands, such as Bima Sumbawa, Banggai, Buton, Wakatobi and Flores Islands. Some of them bring their families and stay at Barrang Lompo, which means the island has become quite crowded.

60 processors used to be on the island, now there are much fewer (reportedly only 4);

the dominant holothurian species collected used to be *Actinopyga* sp. and *Thelenota anax* (not observed in 2016); however, on this recent visit, the dominant species had changed and varied between processors. At one processor, *Holothuria lessoni* (formerly *H. scabra* var. *versicolor*) was predominant, while *Stichopus* sp. (probably *S. naso*) was predominant at another processor.

processing used not include the first cooking onboard, as it does now.

Previously, all of the streets on the island were covered with sun-drying sea cucumbers on blue plastic sheets. Now the roads are sealed and heavily used by numerous motorbikes.

In conclusion, it appears that such repeated surveys over a long time period are useful. This example shows that changes are evident in the fishing-processing-marketing chain for sea cucumbers in Sulawesi. It will be interesting to re-examine the activities in the other islands where such activities have been described in the past, such as Wakatobi (Moore 1998) and Pulau Misa (Navarro et al. 2014).

At the larger scale of Indonesia, Choo (2008) refers to 38 species, and the recent synthesis by Setyastuti and Purwati (2015), based on sampling at four sites in Indonesia, indicates 33 species, which have been taxonomically confirmed. This list does not include *Stichopus naso* but several *Stichopus* spp. are listed, and this genus deserves further investigation.

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Potential symbiosis between the bathyal sea cucumber *Orphnurgus* sp. (Elasipodida, Deimatidae) and the amphipod crustacean *Adeliella* sp. (Gammaridea, Lysianassoidea) in the western tropical Pacific

Laure Corbari,[1,] Chantal Conand[2,3] and Jean Claude Sorbe[4]*

Sea cucumbers are known to harbour a large number of parasitic or commensal organisms, including gastropods, worms, crustaceans, echinoderms and fishes (Eeckhaut et al. 2004; Caulier 2016 (see PhD abstract in this issue); Purcell et al. 2016). More than 200 species are currently recognised as commercial sea cucumbers. Bathyal and abyssal species are also important hosts (Billet 1988; Thurston et al. 1987). Crustaceans associated with deep-sea cucumbers, ectobiontes or endobiontes, belong to different groups (Billet 1988; Thurston et al. 1987). Among the amphipods mentioned as holothurian endobionts, several associations have already been described: *Valettia hystrix* Thurston, 1989 (Valettidae) in the posterior intestine and cloaca of two deep elasipod holothurians at Porcupine Seabight, northeast Atlantic, *Deima validum validum* and *Oneirophanta mutabilis mutabilis* Théel, 1879 (Billett 1988; Thurston 1989). *O. mutabilis* generally harbours five to seven amphipods per digestive tract.

During several expeditions conducted since 1976 by the Muséum National d'Histoire Naturelle (Paris) in the tropical west-Pacific region, numerous benthic samplings (Warén dredge, beam trawl) were carried out on bathyal bottoms in order to inventory the biodiversity of these environments. Among these multiple operations, six trawls carried out with a beam trawl made it possible to collect 15 Lysianassoidea amphipods, probably belonging to a new species in this group. Two of these specimens were directly observed onboard, housed in the coelomic cavity of elasipod holothurians, as evidenced by the photographs of the specimen from station CP1836: one taken onboard immediately after fishing (Fig. 1A), and another after about 15 years of storage in alcohol (Fig. 1B).

The two host sea cucumbers were fished at two geographically distinct bathyal stations, but at similar depths, one in the Solomon Sea (SALOMON 1 campaign, 5 October 2001, station CP1836, 439–486 m, mud), the other north of New Hanover (KAVIENG campaign, 1 September 2014, station CP4448, 564–743 m, mud with fragments of sunken wood). These two small (5–6 cm) specimens probably belong to the same species and are characterised by a similar general morphology, relatively short and few podia, and a longitudinal median line on their ventral surface. Y. Samyn and M. Sibuet (pers. comm.)

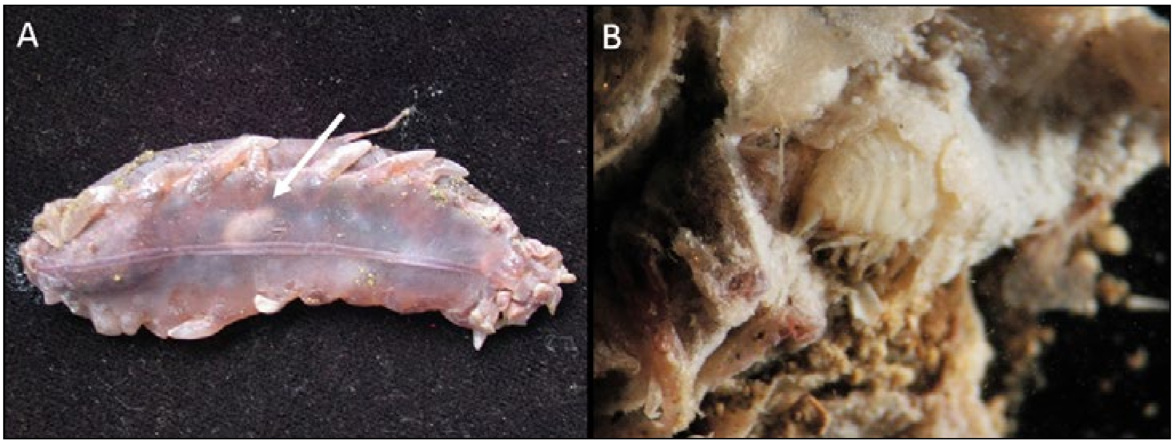

Figure 1. A: Silhouette of an amphipod (indicated by an arrow) seen through the integuments of a sea cucumber (Elasipodida) at the time of collection (station CP1836) (image: A. Warén). B: Amphipod (Lysianassoidea, genus *Adeliella*) partially cleared of the integuments of the same sea cucumber after 15 years of preservation in alcohol. (image: L. Corbari)

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suggest that they belong to the family Deimatidae, which comprises the three genera *Deima*, *Oneirophanta* and *Orphnurgus*. According to Paulay (2015), nine species are currently recognised for this deep-sea genus (174–1301 m), several species having been described from the tropical Pacific, others from the Indian Ocean (Thandar 2015). Pawson (2002) described *O. dorisae* from New Zealand and gave an identification key to all known species. The study of the spicules and anatomical characters of the specimens herein examined may help to clarify their identification.

Of the 15 amphipod specimens from various trawls carried out in the bathyal area (260–865 m) of the western tropical Pacific, only 2 were undoubtedly sea cucumber endobionts. The others were collected in the trawl cod-end, suggesting that they are occasional symbionts that can also have an independent benthic life. According to their morphology, all of these specimens belong to the same species within the Lysianassoidea superfamily. The detailed characteristics of their morphology (under study) reveal that they undoubtedly belong to the genus *Adeliella* described by Nicholls (1938). According to the World Register for Marine Species database (2016), the genus *Adeliella* currently contains four species that have been collected by trawling or in baited traps. They are generally regarded as scavengers and have so far never been mentioned in symbiosis with sea cucumbers.

Figure 2. *Adeliella* sp. (Amphipoda Lysianassoidea, specimen MNHN-IU-2015-262) collected in the Solomon Sea (station CP4338, MADEEP oceanographic campaign, 410–614 m), and photographed after storage in alcohol. (Image: I. Frutos)

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Field observations on the regeneration in *Synapta maculata* (Holothuroidea: Synaptidae)

Philippe Bourjon[1]

Introduction

The order Apodida does not include fissiparous sea cucumber species but at least some species are known to exhibit autotomy and regeneration (Emson and Wilkie 1980). Such capacity has been experimentally studied in *Leptosynapta* Verrill, 1967 (Pearse 1909; Smith 1971a, b; Gibson and Burke 1983). Transversal section experiments reveal that anterior parts regenerate a smaller but functionally complete animal (Smith 1971a), whereas posterior parts never survive (Smith 1971a; Gibson and Burke 1983). Autotomy has been induced in *Synapta maculata* Chamisso and Eysenhardt, 1821 (Domantay 1931), but field observations of the anterior part regenerating a posterior part are uncommon. This communication reports on observations made on a fringing reef at Reunion Island.

Material and methods

An anterior part of a *Synapta maculata* individual regenerating a posterior part was observed *in situ* on 10 September 2016 on the fringing reef of L'Ermitage at Reunion Island (21°07'S, 55°32'E). The specimen was found under a broken slab of concrete, 3 m from the beach and at a depth of 50 cm. The regeneration progress was monitored over seven weeks. Measurements were taken after moving the individual to a nearby sandy area without rubble. When the animal was crawling on a flat surface, its body was roughly straight, and there were very little contraction. After measurements were made, the individual *Synapta maculata* was placed back under its shelter. Photographs were taken during each monitoring session.

The specimen always stayed under the same shelter where it was first found. It was monitored until its appearance and length became similar to young individuals present at the site, and was indistinguishable from others like it.

Results

When the specimen of *Synapta maculata* was found, the loss of its posterior part seemed to have occurred rather recently because its posterior extremity showed petal-like pieces of tegument opening and closing regularly (Fig. 1 a, b, c). The body had the same appearance as whole individuals on the reef, apart from its posterior part (Fig. 1c), whose coloration was semi-transparent, whitish to pale or yellowish grey with a low density of ochre heaps of spicules, and which exhibited a succession of prominent bulging warts. The anterior part was typically wrinkled, yellow-brown longitudinally, with

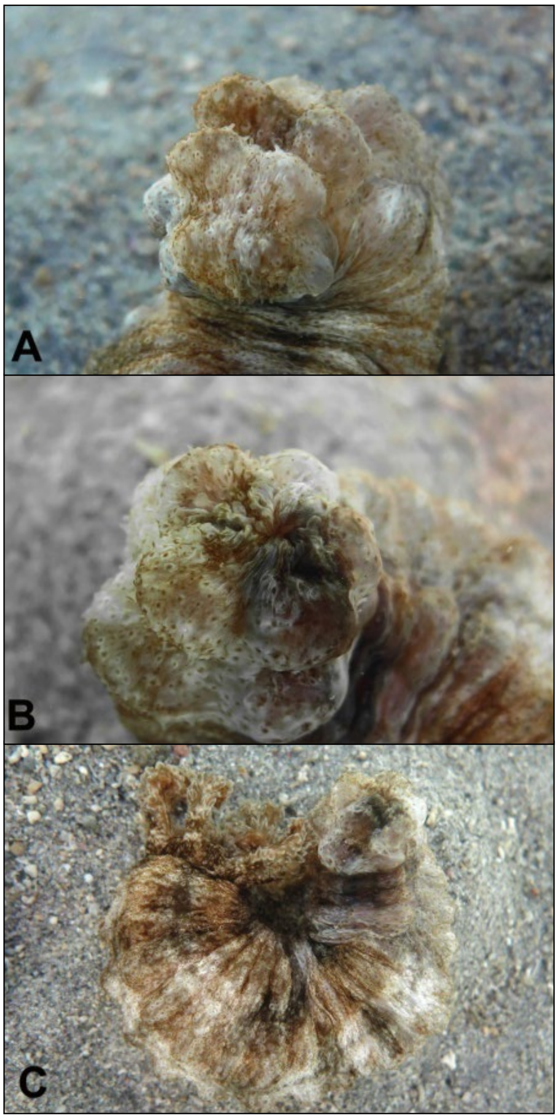

Figure 1. Anterior part of *Synapta maculata* regenerating a posterior part (observed on 10 September 2016). A: Petal-like pieces of tegument "opened" at the posterior extremity at 09.46 am. B: Posterior extremity closed at 09.47. C: Posterior extremity opened again a few seconds later.

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white and brown stripes and transversal dark grey blotches, and exhibited dense patches of spicules. No measurement of the non-contracted body could be done that day, as the individual kept a C-shape posture and did not attempt to crawl. Its length in this contracted posture was about 9.5 cm and its diameter was 3.5 cm; the posterior part was around a quarter of the length of the body (Fig. 1c).

During the following weeks, the specimen was identically characterised by a warty and greyish posterior part while the anterior part was wrinkled and yellowish (Figs. 2 and 3a). The posterior part remained more or less warty even when its colouration became the same as the anterior part. This feature was no longer obvious on 5 November 2016 and, therefore, monitoring ceased on that day (Fig. 3b).

On 17 September 2016, its length of the *Synapta maculata* individual when it was crawling was about 20 cm (Fig. 3a). From 17 September to 5 November, it increased in length regularly by an average of 0.42 cm by day, with the specimen reaching 41.00 cm when the observations ceased (Fig. 3b). An increase in the daily average length was noticed during this period: increasing from 0.30 cm day^{-1} between 17 September and 12 October (26 days), to 0.54 cm day-1 between 12 October and 5 November (24 days).

Discussion

The quantitative data provided here are solely for informational purposes, as the high stretching capacity of this species prevented an accurate estimation of length in the field. Therefore, the change in the ratio of the anterior and posterior parts of the body length based on their characteristic appearances could no longer be estimated. Moreover, the cut on the body seemed to have occurred recently when the specimen was found. The posterior part of the body, close to the wound and equivalent to about 25% of the total body length, was covered with warts. This suggests that this feature could be a post-traumatic response that was rapidly exhibited by the tegument tissues of the anterior part bordering on the wound, as the warts can appear in stressful conditions in this species (Domantay 1953). Inasmuch as this response was also exhibited by the regenerating part during seven weeks, the warty appearance cannot be considered as a reliable characteristic to estimate the growth of this part because it seems impossible to perceive the precise localisation of the transection.

The almost unchanging warty morphology of the posterior part during the observation period suggests that the regenerating part exhibited a long post-traumatic stress period. This may be a way to recognise a regenerating individual in the field.

Figure 2. *Synapta maculata*: warty and greyish posterior part, and an anterior part that is wrinkled and yellowish (observed on 17 September 2016).

Figure 3. Regeneration of *Synapta maculata*.
A - 24 Sept. 2016: The specimen was about 20-cm long. The posterior part was still greyish and warty.
B - 5 Nov. 2016: The specimen measured about 41 cm. The posterior part was, henceforth, approximately similar to the anterior part.

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Biodiversity in holothurians at Reunion Island: Two previously undescribed sea cucumbers species

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Abstract

We present two sea cucumber species discovered at Reunion Island between 2014 and 2016: *Stichopus* sp. and *Holothuria* (*Stauropora*) sp. Because the discoveries occurred within a marine protected area, no specimens were collected. *Stichopus* sp. Specimens were confirmed by DNA sequencing from non-lethal authorised tissue samples; *Holothuria* sp. specimens were determined from photographs with the usual reservations about photograph-based identifications. One adult and two juveniles of *Stichopus* sp. were found, along with two specimens of *Holothuria* (*Stauropora*) sp. Awaiting for type specimens that will allow a conventional description, this communication presents their main morphological characteristics in the field and from photographs, and observations on habitats and behaviour. Photographs of these specimens illustrate some aspects of the descriptions.

Introduction

Two undescribed sea cucumber species were discovered by the authors on the west coast of Reunion Island between 2014 and 2016: *Stichopus* sp. and *Holothuria* (*Stauropora*) sp. No specimens were collected, however, because the discoveries occurred within the perimeter of the marine protected area “Réserve Naturelle Marine de La Réunion (GIP-RNMR)”. Nevertheless, authorised non-lethal tissue sampling allowed DNA sequencing for *Stichopus* sp., while the identification of *Holothuria* (*Stauropora*) sp. was based on photographs. The most recent inventory of holothurians of Reunion Island includes 44 species (Conand et al. 2016), an important increase since the previous one, which included 37 species (Conand et al. 2010). The holothurians at Reunion belong to 5 families, the Stichopodidae with 5 species, and Holothuriidae with 30 species, including the two undescribed species presented here.

Awaiting the collection of type specimens, the present article provides information on the main external morphological characteristics of these two species in the field and from photographs, and on their habitat and some patterns of behaviour. Photographs of these specimens illustrate some aspects of this information.

Material and methods

Observations were made on the Saint Gilles–La Saline Reef (also known as L’Ermitage Reef), on the west coast of Reunion Island (21°07’S and 55°32’E). Saint Gilles–La Saline Reef is 7.6 km long, its maximum width is 500 m, and its maximum depth is less than 2 m at high tide. It is divided in two parts (northern and southern) by L’Ermitage Pass. This reef is included within the perimeter of the marine protected area Réserve Naturelle Marine de La Réunion (GIP-RNMR). One of the observers holds a non-lethal tissue sampling authorisation approved by the Scientific Council of the RNMR.

Observations were made by snorkelling near the surf zone. For underwater exploration, each observer had underwater photography equipment, a ruler, magnifying glass, and a small container for tissue samples that could be made in situ. Tissue sampling equipment was left on shore.

Two specimens of *Holothuria* (*Stauropora*) sp. were observed, the first in the southern part of the reef on 20 July 2014, the second in its northern part on 2 March 2016. No tissue sampling was done because the tegument of the individuals seemed too thin to withstand a cut, but many pictures of the second individual were taken. Three specimens of *Stichopus* sp. were found in the same area in the northern part of L’Ermitage Pass, the first one on 26 September 2015, the second one on 25 November 2015, and the third on 10 January 2016. The first one bore two wounds with white tegument strips trailing out from the body, one on the left side, and the second on the trivium (visible in Fig. 1 a, b). These strips were collected *in situ* with a dive knife because the manual transportation to the shore of a Stichopodidae would have been hazardous for the specimen, considering how easily the tissue of some species in this family can disintegrate. The sample was immediately brought to shore and preserved in 90%

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ethanol. The two other individuals were identified from photographs. All specimens were observed in the morning.

Identifications were made by F. Michonneau (Division of Invertebrate Zoology, Florida Museum of Natural History, FLMNH). The status of undescribed species of *Stichopus* sp., established from DNA sequencing, was confirmed by G. Paulay (Curator of the Division of Invertebrate Zoology, FLMNH). Identification of *Holothuria* (*Stauropora*) sp. was based on photographs with the usual reservations regarding photograph-based determinations.

Information on the external morphology of two specimens is developed from field observations and photographs.

Results

Holothuria (Stauropora) sp.

Aspects of the living animal. Length slightly contracted: around 9.0 cm; mid-body width: 2.5 cm. Bivium blue-grey locally smoky (Fig.1a). Trivium uniformly pale grey. A multitude of minute dark red-brown dots irregularly distributed on the bivium (not visible on photographs). Trivium flattened. Body wall flexible, thin and wrinkled. Two kinds of papillae on the bivium. The first ones high, conical, with a broad whitish base; distal extremities translucent and sprinkled with minute white dots. The second ones smaller; their base brown marked with black dots up to two-thirds of the cone, the tip being whitish. The two kinds of papillae irregularly distributed. Ventral podia long and thin. Stems translucent pale grey with minute white dots, topped by a white disc. Interambulacral areas narrow, without podia. Oral tentacles apparently peltate; colour yellowish-white. Mouth surrounded by two collars of papillae. Cloacal orifice terminal and circular, surrounded by a collar of short whitish papillae. Cuvieran tubules few but with large diameter, rather quickly expelled; colour translucent bluish (Fig. 1b).

The 8.5-cm long second specimen exhibited the same characteristics with two exceptions: a broad white blotch on the bivium slightly behind the first half of the body, and a thin white anterior extremity (Fig. 2a). This extremity, whose diameter was distinctly smaller than that of the preceding body part, suggests a regeneration process. This hypothesis is strengthened by the nipple-like aspect of this part when the body was fully contracted (Fig. 2b). This species could, therefore, reproduce asexually.

Habitat. The two specimens were found in areas of strong hydrodynamic conditions near the surf zone. The habitat of the first one is characterised by a sandy-detritus substrate, including mainly dead coral rubble. The habitat of the second one includes necrotic hard corals. The sandy areas between these corals are cluttered with dead coral rubble, and the two specimens observed were hidden under this rubble. The first one was lying on the substrate whereas the second one was fixed on the underside of slab-shaped debris.

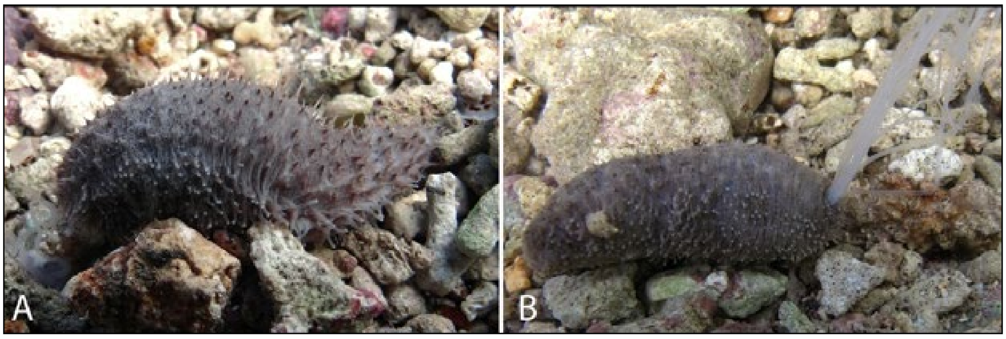

Figure 1. First discovered individual of *Holothuria* (*Stauropora*) sp.
A. Bivium; B. Trivium and Cuvieran tubules.

Figure 2. Second individual of *Holothuria* (*Stauropora*) sp. A. Bivium; B. Nipple-like protrusion on the anterior part, suggesting a regeneration process; C. Cryptic behaviour: attempting to hide within a little arch on the underside of a piece of coral rubble.

Behaviour. The second specimen was observed for one hour, which allowed the sighting of a particular cryptic behaviour. When discovered it immediately tried to hide into one of the holes present on the underside of the rubble. Unsuccessful in its attempt, it painfully got through a little arch, grazing its tegument after one try to move backwards. It was then placed by the observer on one side of the rubble and it got away into another arch (Fig. 2c). When placed on the sandy substrate, it began burying itself. Cuvieran tubules were not expelled by this individual.

Remarks. Except for short papillae and a dorsally arched body, this specimen corresponds to the subgenus *Holothuria* (*Stauropora*) as described by Rowe (1969), considering what can be observed in the field (tentacles could not be counted):

> "Tentacles 18–30, pedicels in three distinct rows on the ventral flattened surface, papillae small, irregularly arranged dorsally, a 'collar' of papillae sometimes present around the base of the tentacles; body wall soft, not very thick, usually about 1 (1–2) mm; body with flattened 'sole'-like ventral side, arched dorsally; size small, up to 100 (rarely 125) mm long..." (Rowe 1969).

This subgenus presently comprises 13 accepted species (Paulay 2015).

Stichopus sp.

Aspects of the living animal. Length slightly contracted: 21 cm (Fig. 3a); mid-body width: 7 cm. Background colour of the bivium more or less pale or yellowish creamy white with numerous small ochre-coloured spots and diffuse areas mixing pink and dark grey on top and sides of the body. Trivium uniformly pale grey (Fig. 3b). Body quadrangular in cross-section, tapering at the two extremities. Trivium flattened. Body wall thick. Transversal folds present on the dorsal side. Big prominent papillae on dorso-lateral and ventro-lateral edges; colour of papillae mostly pinkish with short red lines forming concentric circles from base to summit. Numerous shorter pinkish papillae between edges. All papillae with white bases and translucent distal tips. Papillae irregularly distributed, even on edges. Tube feet long, rather thick and irregularly scattered only along ambulacral areas. Stems pale grey with minute black and white dots and white distal disk. Interambulacral areas relatively wide. Mouth apparently subterminal. Tentacles greyish and apparently peltate. Anus terminal. Cloacal orifice surrounded by a collar of very short bumplike papillae.

The first specimen is probably an adult. The two juveniles found in the same area are, respectively, 10.5 cm and 7.2 cm long (Fig. 4a and b), both slightly contracted. Because the first specimen was observed on 25 November 2015 and the second one, clearly smaller, on 10 January 2016, they cannot be the same individual. The adult's colours are paler in the juveniles. The background creamy white colour becomes pure white, with pinkish areas. Small ochre-coloured spots of the adult are grey in the smallest juvenile, and grey or pink in the bigger juvenile. The trivium is pinkish white (Fig. 4c). Prominent papillae are fewer than in the adult, and principally distributed on the four edges. The cloacal orifice was observed successively in terminal and dorsal positions in the bigger juvenile.

Figure 3. First discovered individual of *Stichopus* sp. A. Bivium; B. Trivium; C. Fully contracted body.

Figure 4. Two juveniles of *Stichopus* sp. A. First individual; B. Second individual; C. Trivium of the first individual.

Habitat. The three specimens were found in an area of strong hydrodynamic conditions near the surf zone. Their habitat is characterised by a sandy-detritus substrate that includes dead coral rubble.

Behaviour. The two juveniles displayed cryptic behaviour, and were hidden under dead coral blocks, while the adult specimen was fully exposed on the substrate among the blocks. None of the three specimens reacted to brief handling by altering their superficial tissues. Nevertheless, the two pieces of tegument removed from the adult specimen quickly became a translucent viscous substance.

Remarks. External morphology features of species of the genus *Stichopus* Brandt, 1835 make accurate identifications difficult in the field because they can be similar interspecifically and variable intraspecifically (Massin et al. 2002). Compared with stichopodid species recorded from La Reunion, these three specimens looked like individuals of the *Stichopus monotuberculatus* complex at first glance, particularly because they exhibited prominent papillae with coloured rings. *S. monotuberculatus* displays highly variable colour patterns (Byrne et al. 2010). One of them is creamy white with red and grey or black patches on the bivium, which make it relatively similar to that of *Stichopus* sp. specimens from La Reunion, but the trivium of *S. monotuberculatus* is longitudinally blotched with grey to black markings while it is uniformly whitish to pale grey in our specimens. Regarding the general shape of the body, *S. monotuberculatus* does not taper at the posterior extremity; the quasi-spherical shape of *Stichopus* sp. when fully contracted (Fig. 3c) is not typical in *S. monotuberculatus*, suggesting a thinner body wall. Moreover, *S. monotuberculatus* is active at night (Byrne et al. 2010) and hidden under rubble during the day, whereas the adult specimen of *Stichopus* sp. was exposed on the substrate at 10:30.

This species is genetically close to an undescribed *Stichopus* sp. from Hawaii (Godwin & Bolick, 2006) and could be the same (F. Michonneau pers. comm.). The genus *Stichopus* presently comprises 14 accepted species (Paulay and Hanson 2013).

The fact that sea cucumbers are not commercially exploited in Reunion Island (Conand and Frouin, *in* Conand and Muthiga 2007) probably facilitated the preservation of these species. However, their shallow coastal habitat is likely to make them vulnerable to anthropogenic pressures, particularly in a place as densely populated as Reunion's western coast.

Acknowledgements

We warmly thank Chantal Conand for her long-term encouragements and help, and F. Michonneau for his species determinations. We also thank the Reunion Island Direction de l'Environnement, de l'Aménagement et du Logement, along with the RNMR Scientific Council for the tissue sampling authorisation granted to the first author.

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Holothuroidea species found in Belizean waters

Janel McNab[1] and Arlenie Rogers[2]

Introduction

Most sea cucumber fisheries are ineffectively managed, leading to declining stocks and potentially destroying the resilience of such important fisheries (Purcell et al. 2013). In their global analysis of drivers of sea cucumber overfishing, Purcell et al. (2013) revealed an alarmingly high incidence of overexploitation and depletion of sea cucumber stocks, particularly in the Indo-Pacific. In this region, much of the fishing of tropical sea cucumbers occurs on coral reefs and lagoons, which are under particular threat from global impacts such as climate change and ocean acidification. They note the lack of data on basic biological parameters of most species as a fundamental barrier to improved management of these fisheries and many species being threatened with local extinction.

The demand for sea cucumbers in the Asia and Pacific regions, especially as a food delicacy is the main driver for the overharvesting of various species. The highest number of species is exploited in the Asia and Pacific regions, where Indonesia is the world's top producer of Holothuroidea from the capture fishery. Indonesia, together with the Philippines produced an annual average of 47% of the world's Holothuroidea landings, comprising an annual average of 2,572 t (wet weight) between 2000 and 2005 (Choo 2008). A few sea cucumber species within the regions of Mexico, Central America and South America are commercially exploited; with most species lacking biological and ecological studies that would otherwise guide management plans for sustainable resource use (Toral-Granda 2008). Only Cuba, Peru, Chile, Mexico and Ecuador have regulated fishing activities, with Mexico and Ecuador focusing mostly on *Isostichopus fuscus*, and Mexico, to a lesser degree, on *Parastichopus parvimensis* (Toral-Granda 2008). Recently, however, other species such as *Holothuria fuscocinerea, H. inornata* (Benítez-Villalobos et al. 2013), *H. mexicana*, *H. floridana*, *Astichopus multifidus* and *Isostichopus badionotus* (Solís-Marín and Honey-Escandón 2014). In Belize, *H. mexicana* and *I. badionotus* have been legally fished since 2009 (Perez and Garcia 2012).

Of the 52 commercially exploited species reported by Purcell et al. (2012) most are tropical and subtropical species belonging to the families Holothuroidea and Stichopodidae. The systematic character of these or any other holothurian can be identified with the use of spicules (Pawson and Barraclough-Fell 1965). Toral-Granda (2005) suggests that using spicules can be particularly useful in identifying species illegally traded as, for example, when Galapagos *Isostichopus fuscus* had been impounded in the Galapagos National Park Service. The use of spicules in holothuroid identification is useful because some species have similar morphology and once processed, some species may look alike. Likewise, changes in morphology also occur during somatic growth from juvenile to adult stages (Cutress 1996). Depending on the species, these spicules constitute about 3–70% of the dry weight of the holothuroid body wall (Pawson et al. 2010) and may show a great variety of forms.

Although there have been only few arrests in the sea cucumber fishery in Belize, illegal harvests exist (Perez and Garcia 2012). The objective of this study was to confirm the identification of five species by spicule morphology. This study will fill a small gap, helping to create baseline information about sea cucumber species that may have market potential.

Methodology

Six species of sea cucumbers from southern Belizean waters were handpicked by free divers in the main lagoon between the Belize Barrier Reef, and near the mainland from Independence Village and Punta Gorda Town, between 2009 and 2015. Each individual was photographed, its morphological features studied, and regional geographic range noted. Individuals were subsequently preserved in methanol. Spicules were prepared using established methodologies (Purcell et al. 2012). The spicules were photographed and the species identified using identification guides by Purcell et al. (2012) and Pawson et al. (2010).

Results

In all samples, the spicules remained distinguishable although they were in a preserved state. The species and their extracted spicules are presented below.

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Holothuria mexicana (juvenile) (Fig. 1) exhibited the following spicule shapes (Fig. 2): ventral body wall: biscuit (A); dorsal body wall: developing biscuit shape (B); podia: developing biscuit shape (C and D); mouth: developing biscuit shape (E and F); anus: developing biscuit shape (G); table (H); biscuit shape (I).

Isostichopus badionotus (Fig. 3) exhibited the following spicule shapes (Fig. 4): ventral body wall: rod (A), table (B), C-shaped (C); dorsal body wall: C-shaped (D), table (E); podia: biscuit shape (F), perforated plate (G), rod (H); ventral mouth area: table (I); ventral anus area: table (J).

Actinopgya agassizii (Fig. 5) exhibited the following spicules (Fig. 6): ventral body wall: rosette (A); dorsal body wall: rosette (B); mouth: rod (C); anus: rosette (D).

Figure 1. Live specimen of *Holothuria mexicana* (image: Arlenie Rogers)

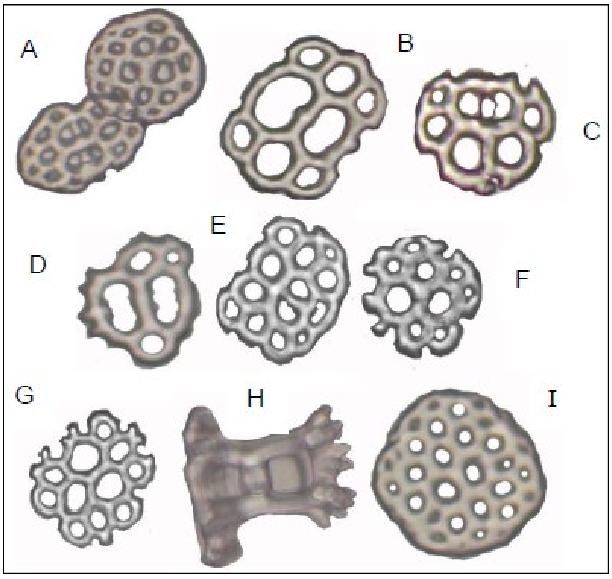

Figure 2. Microscopic images of *Holothuria mexicana* spicules. (image: Janel McNab)

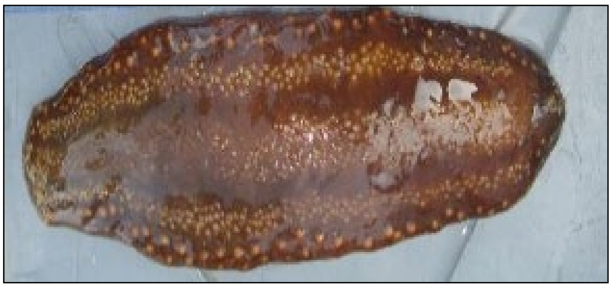

Figure 3. Live specimen of *Isostichopus badionotus.* (image: Arlenie Rogers)

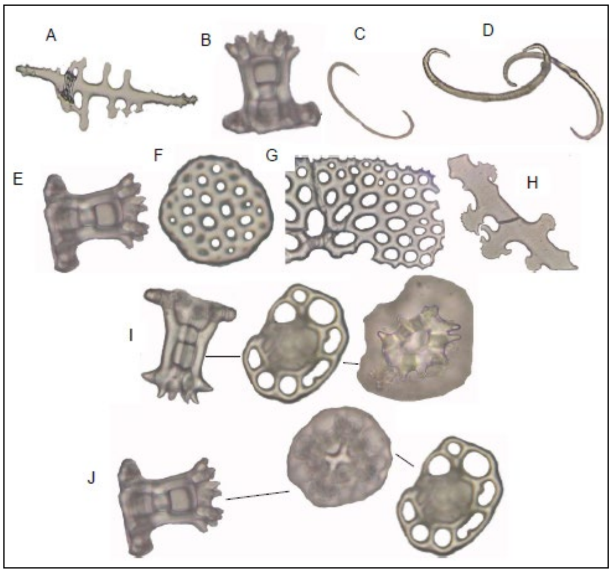

Figure 4. Microscopic images of *Isostichopus badionotus* spicules. (image: Janel McNab)

Figure 5. Live specimen of *Actinopgya agassizii*. (image: Arlenie Rogers)

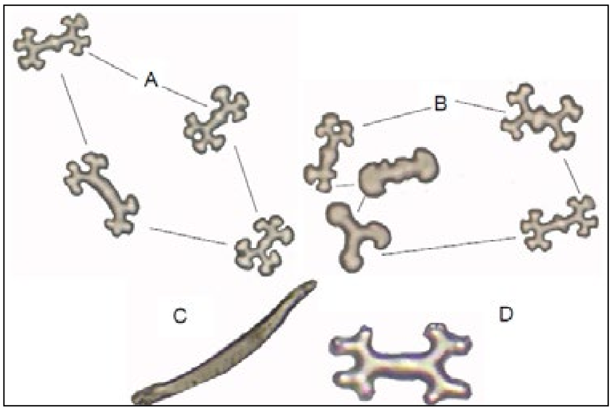

Figure 6. Microscopic images of *Actinopgya agassizii* spicules. (image: Janel McNab)

Holothuria mexicana (Fig. 7) exhibited the following spicules (Fig. 8): ventral body wall: biscuit shape (A); dorsal body wall: table (B), biscuit shape (C), biscuit shape (D); podia: biscuit shape (E); mouth area: biscuit shape (F), rod (G); anus: rod (H), biscuit shape (I & J), table (K).

Astichopus multifidus (Fig. 9) exhibited the following spicules (Fig. 10): ventral body wall: O-shape (A), C-shape (B), miliary granules (C), rod (D); dorsal body wall: O-shape (E), C-shape (F), miliary granules (G); mouth: rod (H), C-shape (I), miliary granules (J); anus: C-shape (K) and miliary granules (L).

Holthuria thomasi (Fig. 11) exhibited the following spicules (Fig. 12): ventral body wall: smooth button (A), table (B); dorsal body wall: smooth button (C), rod (D), table (E); podia: perforated plate (F) and smooth button (G); mouth: smooth button (H) and table (I); anus: rod (J) and table (K).

Figure 7. Live specimen of *Holothuria mexicana.* (image: Arlenie Rogers)

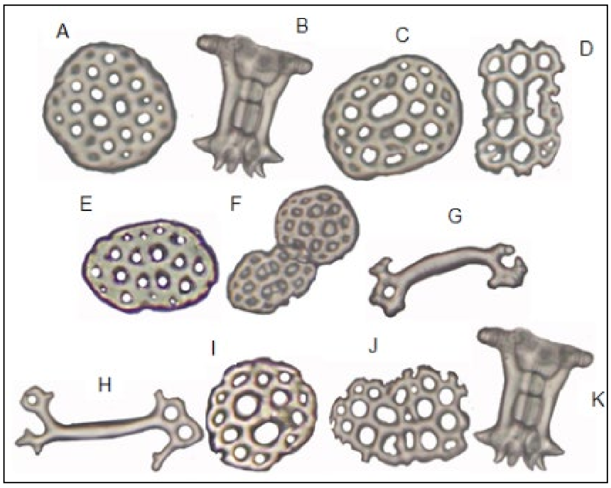

Figure 8. Microscopic images of *Holothuria mexicana* spicules. (image: Janel McNab)

Figure 9. Live specimen of *Astichopus multifidus.* (image: Jane Salazar)

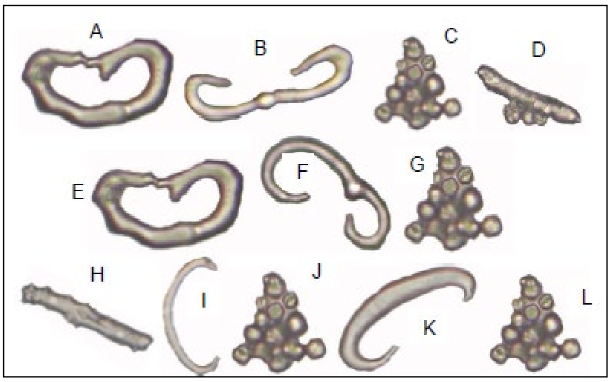

Figure 10. Microscopic images of *Astichopus multifidus* spicules. (image: Janel McNab)

Figure 11. Live specimen of *Holthuria thomasi*. (image: Arlenie Rogers)

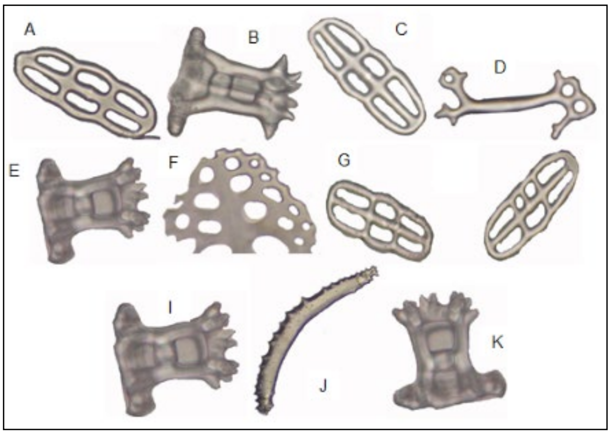

Figure 12. Microscopic images of *Holthuria thomasi* spicules. (image: Janel McNab)

Discussion

The use of spicules for identification is a rather simple method and can be completed within a few hours (Toral-Granda 2006). This is especially useful in developing countries, such as Belize, that lack expertise and technology to complete other identification methods such as the use of molecular analyses.

Although only *Holothuria mexicana* and *Isostichopus badionotus* are fished for in Belize, other commercial species can be found there, including *Actinopyga multifidus* and *A. agassizii* and *H. thomasi.* These results will hopefully provide useful information on the species found in Belize and aid in the identification of harvested species.

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Observation of mass recruitment of juvenile dendrochirotids on coral reefs of Sulawesi, Indonesia

Syafyudin Yusuf[1] and Ambo Tuwo[1]

Observation

Location and method: Westward fringing reef of Samalona Island, 05°07′19.76″S and 119°20′24.94″E. Samalona Island is a small island about 5 km from the city of Makassar on southwest Sulawesi in the Spermonde Archipelago, Indonesia. Scuba diving depth: 5 m.

Date: 5 March 2011, end of the rainy season; the seawater was rich in nutrients from the river.

Habitat characteristics: Juveniles were found living on coral reef substrate with turf and coralline algae. The coral reef substrate coverage consisted of hard corals (18.8%) and dead coral with rubble (71.1%), with other organisms, including holothurians (10.1%).

Juvenile characteristics: Long white tentacles, longer than the bodies; body length 6–8 mm; colour: dark green skin with orange lines. (Adults are dispersed and cryptic; only the long tentacles make them visible.)

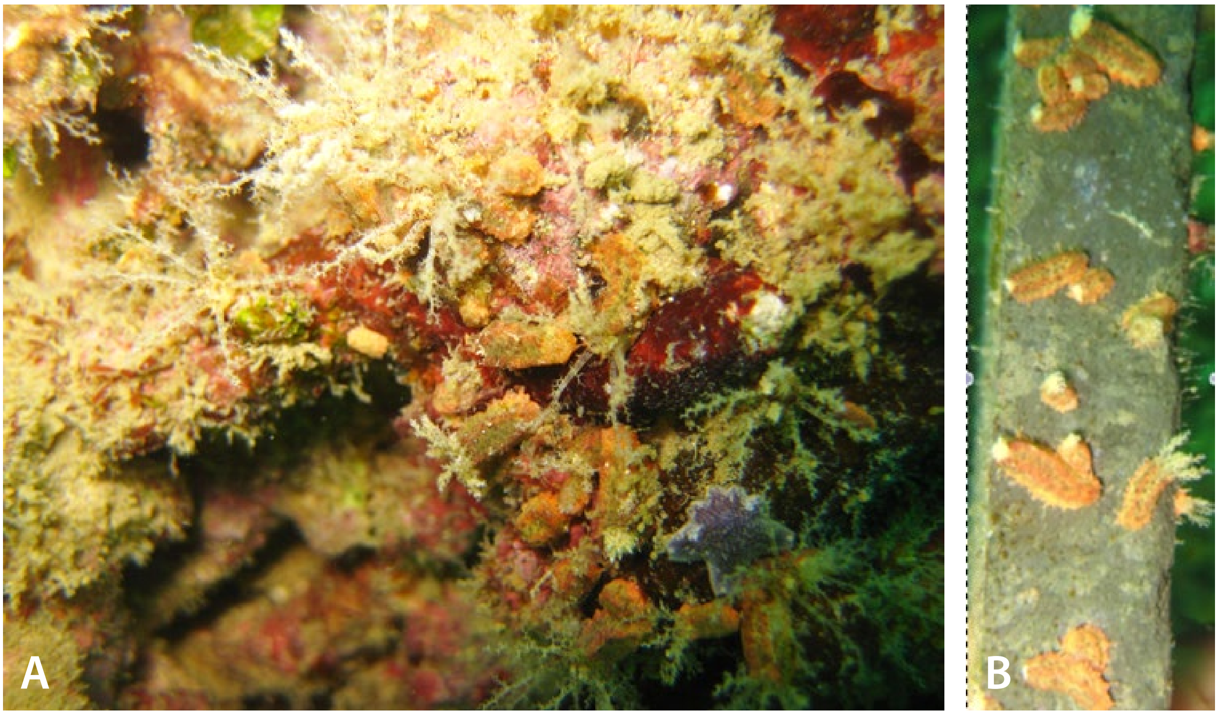

Figure 1. A: adult and juvenile dendrochirotids; B: close up of settled juveniles (6–8 mm).

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An aquarium trade dendrochirotid holothurian, *Pseudocolochirus* sp., processed into *teripang* in Sulawesi, Indonesia

Syafyudin Yusuf,[1] Ambo Tuwo[1], Joeharnani Tresnati[2] and Chantal Conand[3,4]

Observation

On 20 December 2016, during a visit with sea cucumber processors on Barang Lompo Island, Spermonde Archipelago, southwest Sulawesi, Indonesia (see contribution on p. 99, this issue), several specimens of a species frequently used in the aquarium trade, *Pseudocolochirus* sp., were observed half processed. This species is locally called *teripang kurma*, or date palm tree, given its appearance. The specimens were measured at 8.0, 9.0 and 9.5 cm in length.

Figure 1. Three specimens of *Pseudocolochirus* sp. at the processor shop, at Barang Lompo Island, southwest Sulawesi, Indonesia.

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Report on a stranding of the dendrochirotid sea cucumber *Cladolabes perspicillum* and other echinoderms by a low-pressure induced storm surge on the New South Wales coast, Australia

Maria Byrne,[1] Kennedy Wolfe,[1] Mark O'Loughlin[2] and Ashley Miskelly[3]

Introduction

Mass mortalities of echinoderms occur when substantial numbers of individuals of a population die due to the stress of abiotic factors such as freshwater flooding and heat waves (Lawrence 1996). Some of the most dramatic mass mortalities are those observed following storm-induced wave surges when echinoderms, along with a plethora or other marine plants and animals, are washed up and deposited above the high tide line. These events can have long-lasting impacts for the community and ecosystem, although this has not been well studied (Lawrence 1996).

Following the end of the 2016 El Niño, an east coast low (ECL) pressure system developed along the eastern fringe of Australia, from central Queensland to Tasmania, in June. ECLs are intense low pressure systems that develop along the coast. Sometimes referred to as extratropical cyclones, ECLs cause extreme rainfall, storm force winds, and prolonged and heavy swells. While these events are short-lived (several days), they can have severe consequences for coastal regions. The 2016 ECL (5–6 June) coincided with a king tide, resulting in record-breaking waves (max. 17.7 m) pounding parts of the New South Wales (NSW) coast.

The 2016 ECL removed extensive kelp beds and seagrass habitat, and scoured subtidal rocky reefs, resulting in the deposition of vast amounts of marine plants and animals 2–3 m in height along portions of the coast (Fig. 1a). Mixed among the debris, high on the shore at Silver Beach and Quilbray Bay in Kurnell, Botany Bay near Sydney, were many individuals of the dendrochirotid sea cucumber *Cladolabes* (*Urodemas*) *perspicillum* (Selenka, 1867) and other echinoderms (Fig. 1b, c). Around 70 *C. perspicillum* were washed up over a 40-m stretch of beach. While this is not a mass stranding, we have no data on the population density of this species, so it is not possible to judge what proportion of the population this represents. Over 20 years of observing similar storm event depositions at Kurnell, this was the first observation of this sea cucumber in the jetsam (Miskelly pers. Obs.). This contrasts with the common mass stranding of sea urchins associated with these events (Fig. 1d). Following this particular ECL event, 16 species of sea urchins were observed (Table 1) as well as an asteroid (*Astropecten vappa*) and an ophiuroid (*Ophiarachnella ramsayi*).

Cladolabes perspicillum occurs in seagrass habitats and under boulders, and has been dredged from soft sediment areas (Clark 1946; Cannon and Silver 1986). It is a fairly stout species (150–200 mm in length), and is relatively ovoid in shape (Cannon and Silver 1986). Live specimens contract into a round shape (Fig. 1c) and are often found characteristic of Sydney Harbour. There have been other reports of mass standings in New Zealand by storms of dendrochirotid species (e.g. *Caudina coriacea*, *Colochirus ocnoides*) and for tropical aspidochirotid species (e.g. *Holothuria atra*, *Actinopyga echinites*) by typhoon-generated waves (Kerr 1992; Lawrence 1996).

There have been 25 records of *C. perspicillum* in Sydney Harbour from 1887–1981 (Hutchings et al. 2013), and several records from more northern areas in NSW (Rule et al. 2007). There are, however, no details of the biology and ecology of this species. This paucity of information makes it difficult to suggest what the longer-term impact of the stranding of *C. perspicillum* on the local population and ecosystem might be. As characteristic of dendrochirotids, this species is a suspension feeder and its removal is likely to have an impact on local benthic–pelagic coupling. In contrast, many of the sea urchin species that were washed up have been well studied in the context of their role as algal grazers and ecological engineers. For the urchins, the ECL appears to have not only removed a large proportion of the population of many species, but also their kelp bed habitat (Fig. 1a). This is likely to have changed the local habitat and ecosystem, at least in the short term. The lack of before-storm subtidal images and post-storm monitoring limits what can be said for the urchins.

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[1] University of Sydney, Sydney, NSW, Australia
[2] Museum Victoria, Victoria, Australia
[3] The Sea Urchin Science Centre and Gallery, Kurrajong, NSW, Australia

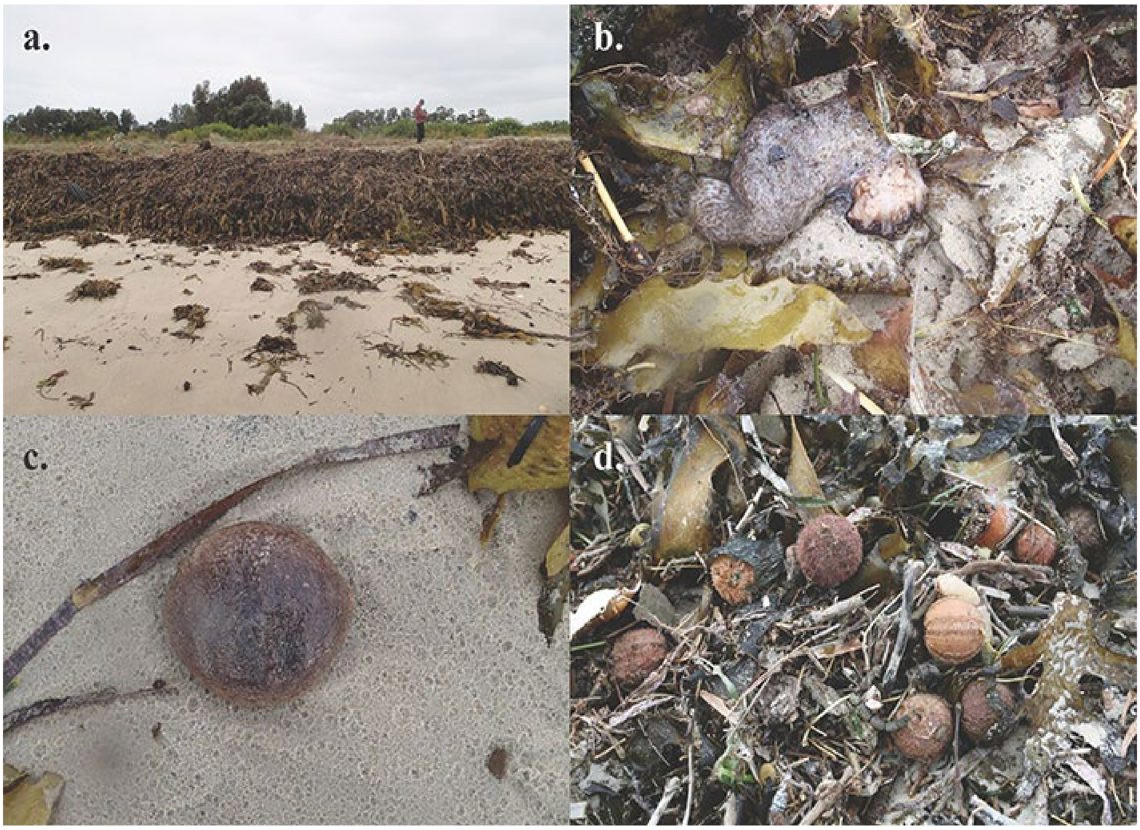

Figure 1. Images of (a) marine debris above the high tide mark at Silver Beach, New South Wales, Australia, and (b and c) *Cladolabes perspicillum*, and (d) echinoids found in the jetsam of the 2016 east coast low pressure system.

Table 1. Species of Echinoidea washed up at Silver Beach, Kurnell, New South Wales during the July 2016 east coast low storm

Family	Species
Brissidae	*Brissus agassizii*
	Rhynobrissus hemiasteroides
Cidaridae	*Phyllacanthus parvispinus*
Diadematidae	*Centrostephanus rodgersii*
Echinometridae	*Heliocidaris erythrogramma*
	Heliocidaris tuberculata
Loveniidae	*Echinocardium cordatum*
	Lovenia elongata
Schizasteridae	*Protenaster australis*
Temnopleuridae	*Holopneustes inflatus*
	Holopneustes purpurascens
	Salmaciella oligopora
	Salmacis sphaeroides
	Temnopleurus alexandri
	Temnoplerus toreumaticus
Toxopneustidae	*Tripneustes gratilla*

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COMMUNICATIONS

III Congreso Latinoamericano de Equinodermos, Costa Rica

by Juan José Alvarado[1,2,] and Chantal Conand*

The third Latin American Echinoderm Congress was organized in San José, Costa Rica, from 18 to 22 July, at the University of Costa Rica, by the Centro de Investigacion en Ciencas del Mar y Limnologia, and by the Biology School, with the coordination of Juan José Alvarado Barrientos, with the help of a local team and a scientific committee from several countries (México, Spain, Brazil, Ecuador and Argentina). Financial support was offered by Institut Français Amérique centrale and Vicerrectoría de Investigación from the University of Costa Rica which are sincerely thanked.

The congress was attended by 74 participants from 13 countries (Spain, USA, Mexico, El Salvador, Costa Rica, Colombia, Ecuador, Chile, Venezuela, Brazil, Uruguay, Argentina and France). There were a total of 93 presentations, 53 oral and 40 posters, with a participation of 37 students (undergraduate and graduate). Four plenary lectures were given: 1) "Recent trends in world sea cucumber fisheries: Captures, markets, management and the problem of the illegal" by Dr Chantal Conand, La Reunion University, France; 2) "Human influence on the population fluctuations of sea urchins: Implications for management of marine ecosystems" by Dr Jose Carlos Hernandez, University of La Laguna, Tenerife, Spain; 3) "Ethical reflections on the use of echinoderms in scientific studies" by Dr Tamara Rubilar, Patagonian National Center, Argentina; and 4) "Molecular studies of echinoderms across the Atlantic-Mediterranean area: From population genetics to gene expression", by Dr Rocío Pérez Pórtela, University of Miami, United States. The proceeding from this congress will be published in a special issue of the Journal of Tropical Biology in 2017. A week before the congress, three courses were developed and held at the Biology School: 1) "Echinodermata physiology" by Dr Tamara Rubilar; 2) "Experimental design in marine ecology" by Dr Jose Carlos Hernandez; and 3) "Application of molecular markers to ecological and evolutionary studies" by Dr Rocio Perez Portela.

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The following presentations focused on sea cucumbers:

- Effects of different diet types on the growth, survival and regeneration of the sea cucumber *Isostichopus fuscus* induced by a transverse cutting experiment.
  Sonnenholzner J.I., Searcy-Bernal R. and Panchana-Orrala M.
- The genus *Synallactes* (Echinodermata: Holothuroidea: Synallactidae) revisited.
  Solís-Marín F.A. and Laguarda-Figueras A.
- Caracterización del ciclo gonádico del pepino de mar café (*Isostichopus fuscus*, Ludwig 1875) en la costa oriental de Baja California.
  Pañola-Madrigal A. and Calderón-Aguilera L.E.
- Phylogeny of echinoderms. including *Xyplopax*.
  Janies D.A.
- Hermafroditismo en *Isostichopus fuscus* (Holothuroidea) en el norte del Golfo de California, México.
  Herrero-Pérezrul M.D., Pañola-Madrigal A., Calderón-Aguilera L.E. and Reyes-Bonilla H.
- Marsupial brooding in the sea cucumber *Cladodactyla crocea* (Lesson, 1830) (Holothuroidea: Cucumariidae) from southwestern Atlantic Ocean.
  Martinez M.I., Alba-Posse J.E. and Penchaszadeh P.E.
- Revisión de los ejemplares de las especies de la familia Cucumariidae Ludwig, 1894 (Holothuroidea: Dendrochirotida) depositados en la Colección Nacional de Equinodermos "Dra. Ma. E. Caso M" del ICML, UNAM.
  Sánchez-Alonzo D.M., Solís-Marín F.A. and Laguarda-Figueras A.
- Revisión taxonómica del género *Paroriza* Hérouard, 1902 (Echinodermata: Holothuroidea: Synallactidae).
  Laguarda-Figueras A., Solís-Marín F.A., Cervantes-Aguilar I.P. and Méndez-Loyola K.
- Morphological phylogeny of the phyllophorid dendrochirotids.
  Martins L., Souto C. and Tavares M.
- Avances sobre la acuicultura del pepino de mar *Isostichopus fuscus* en Ecuador con visión hacia la prepoblación.
  Sonnenholzer J.

Posters on holothurians were exhibited throughout the congress:

- Inventory of the Echinodermata from Reunion Island, Western Indian Ocean.
  Conand C., Boissin E., Mulochau T., Trentin F. and Ribes S.
- Densidad de equinodermos des littoral rocoso de Punta Amapala y el arrecife de coral de Los Cabanos, El Salvador.
  Segovia J., Ramos F., Guerra G., Lopez G. and Solorzano S.
- Invertebrados asociados a equinodermos en costa des estato Sucre, Venezuela.
  Gomez-Maduro M., Diaz-Diaz O. and Lira C.
- The genus *Synallactes* revisited.
  Solís-Marin F.A. and Laguarda-Figueras A.
- Revision de los ejemplares de las especies de la famila Cucumariidae Ludwig, 1894 deposidados en la Coleccion Nacional de Equinodermos "Dra Ma. E. Caso" del ICML, UNAM
  Sánchez-Alonzo D.M., Solís-Marin F.A. and Laguarda-Figueras A.
- Holothuroideos de Mar Profundo des Caribe y Golfo de Mexico.
  Duran-Gonzalez A., Laguarda-Figueras A., Cervantees-Aguilar P. and Solís-Marin F.A.
- La colecion de Equinodermos des Museo de Zoologia de la Universidad de Costa Rica.
  Alvarado J.J., Chacon I. and Solano-Rivera S.
- First record of Ypsilothuriidae in the south Atlantic.
  Martins L. and Tavares M.
- Caracterizacion des ciclo gonadico del pepino de mar café (*Isostichopus fuscus*, Ludwig 1875) en la costa oriental de Baja California.
  Panola-Madrigal A. and Calderon-Aguilera L.E.
- Hermafroditismo en *Isostichopus fuscus* en el norte del Golfo de California, Mexico.
  Herero-Perezul D., Panola-Madrigal A., Calderon-Aguilera L.E. and Reyes-Bonilla H.
- Marsupial brooding in the sea cucumber *Cladodactyla crocea* (Lesson, 1830) from the southwestern Atlantic Ocean.
  Martinez M., Alba-Posse J.E. and Penchazadeh P.E.
- Comparison of the reproductive cycle of the sea cucumber *Holothuria arguinensis* at three different habitats in the Algarve coast (southern Portugal).
  Marquet N., Conand C., Power D.M., Canario A.V. and Gonzalez-Wanguemert M.

- Distribucion de tallas y reproduccion de *Hemiodema spectabilis* en el Noroeste des Golfo San Matias, Patagonia, Argentina.
  Lazari C., Carignano A., Morsan E., Kroeck M. and Rubilar T.
- Morphological phylogeny of the phyllophotid dendrochirotids.
  Martins L. Souto C. and Tavares M.
- Pruebas de marcaje en los pepinos de mar *Isostichopus fuscus* y *Holothuria theeli* en condiciones de laboratorio.
  Sonnenholzer J., Herrera M.A., Arriega-Ochoa J. and Panchana M.
- Digestive enzymes in gut and respiratory tree of adult sea cucumber *Isostichopus badionotus.*
  Martinez-Milian G. and Olvera-Novoa M.A.

Note:

The national newspaper *La Nacion* published a long article on sea cucumber exploitation "Expertos alertan sobre explotación de pepino de mar", (Experts warn about exploitation of sea cucumbers) following the plenary session.

Communications on holothurians at the Ninth European Echinoderm Conference – Sopot, Poland 2016

Compiled by Thomasz Borszcz (tomaszborszcz@gmail.com)

- The variety of interactions between symbiotic gastropods and echinoderms [talk]
  Dgebuadze and Kantor
- "Cool" sea cucumbers! Biodiversity of Bransfield Strait in Antarctica [poster]
  Cánovas et al.
- Does space matter? Optimizing stocking density of the sea cucumber *Holothuria arguinensis* in tanks [poster]
  Domínguez-Godino and González-Wangüemert
- Sea cucumbers: New marine resource for fisheries and aquaculture in the Mediterranean and the Atlantic [talk]
  González-Wangüemert et al.
- New target species of sea cucumber for aquaculture development in Angola [poster]
  González-Wangüemert et al.

Photo of participants (image: Kajetan Deja).

Conference "Protection and Sustainable Utilization of Sea cucumbers", organized by the Chinese Society of Echinoderm, under the 2016 Annual Conference of Chinese Society for Oceanology and Limnology

Communication by Francisco Solís-Marin

Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México – November 2016

Conference date and location: 6–9 November 2016, Haikou, Hainan Province, China

Principal organizer: Dr Hongsheng Yang, Professor/Deputy Director of Oceanology, Chinese Academy of Sciences.

The conference "Protection and Sustainable Utilization of Sea cucumbers" featured technical sessions and workshops covering nearly all aspects of biology, ecology, physiology, aquaculture and stock enhancement of sea cucumbers, especially *Apostichopus japonicus,* around the world.

Articles published on the web

Sea cucumber makes its way in Nicaragua *(in Spanish)*

During the last three years, the production and export to Asian markets of sea cucumbers have increased sharply in Nicaragua
http://www.laprensa.com.ni/2016/08/02/economia/2077131-pepino-de-mar-impulsa-a-nicaragua

The "espardenya", a treasure of Ibiza

http://www.estorrent.net/en/2016/02/01/the-espardenya-a-treasure-of-ibiza/

Conferences announcements

Welcome to the 16th International Echinoderm Conference, Nagoya, Japan

It is our great pleasure to announce that the 16th International Echinoderm Conference (IEC) will be held in Nagoya, Japan from 28 May to 1 June 2018. This is the second IEC in Japan since the 7th conference was held in Atami in 1990. We have already received inquiries and comments about this conference, for which we are very thankful.

Nagoya is a big city located between two megalopolises, Tokyo and Osaka, and is located in the central part of Japan. The Chubu International Airport is convenient to Nagoya, connected in less than 1 hr by train to the city centre. There are direct flights from many Asian cities, Europe and USA. Nagoya is also connected to Tokyo, Osaka and other cities by Shinkansen (bullet trains, JR system). Therefore, you can easily plan to extend your stay in Japan and visit Tokyo or Kyoto.

The conference will include scientific sessions held in parallel in two or three rooms, poster sessions, as well as plenary sessions in a large hall. These will be held in the Noyori Conference Hall of Nagoya University.

The details of the 16th IEC (First Circular) is scheduled to be announced on the conference webpage in April 2017. For questions and comments, please send an email to the address below.

I look forward to your participation to the 16th IEC in Nagoya, Japan!

Tatsuo Oji (Organizing Committee): oji@num.nagoya-u.ac.jp

World Aquaculture 2017 - June 26-30, 2017 Cape Town International Convention Centre, South Africa

Georgina Robinson and Cathy Hair, session chairs for the Holothurian session

The deadline for abstract submission and for oral or poster presentations at World Aquaculture 2017, which will be held in Cape Town, South Africa from 26–30 June 2017 ended on 1 February 2017. However, it will still be possible to submit revised abstracts up until 30 April 2017. For abstract submission details, please see: http://www.was.org/meetings/default.aspx?code=WA2017

Invited speaker profiles can be viewed at: https://www.was.org/view/world-aquaculture-2017-keynote-speakers.aspx

Tenth WIOMSA Scientific Symposium

The Western Indian Ocean Marine Science Association (WIOMSA), the Institute of Marine Science (IMS) and the Department of Aquatic Sciences and Fisheries [both of the University of Dar es Salaam] and the Tanzania Fisheries Institute are pleased to announce the Tenth WIOMSA Scientific Symposium which will be held in Dar es Salaam on the 30 October–4 November 2017.

The week will be divided into the following components:

1. The Tenth WIOMSA Scientific Symposium – 30 October to 1 November 2017. This component will include: keynote presentations, oral and poster presentations.
2. Specially convened sessions and roundtable discussions – 2 November 2017
3. WIOMSA General Assembly – 3 November 2017
4. Excursions and tours to different places of interest – 4 November 2017

Next North American Echinoderm Conference dates and information

Information provided by Roberta Challener (roberta@nzdiary.com)
Sunday, 9 July 2017: welcome reception in the evening.
Thursday, 13 July 2017: the next NAEC will be held at the College of the Holy Cross in Worcester, MA.

PhD Dissertations

Biological and economic characteristics associated with the body size of commercially important Aspidochirotida sea cucumbers

M. Dumestre (Swire Institute of Marine Science, University of Hong Kong. Email: marielle.dumestre@gmail.com)

As a delicacy, the processed body-wall of sea cucumbers, beche-de-mer, is among the most high-valued of seafood commodities harvested globally to supply, almost exclusively, demand from the Chinese market. Prompted by unsustainable fishing practices targeting more than seventy species, predominantly from the order Aspidochirotida, fisheries management is increasingly being implemented, with mixed success and ongoing difficulties of implementation. Knowledge gaps regarding holothuroids life-history traits are a common constraint, especially due to the dearth of methodologies suitable when studies need to consider their high morphological plasticity. This thesis investigated interdisciplinary aspects of size in holothuroids, from pricing of beche-de-mer to methodological approaches for determining reproduction and growth of *Holothuria leucospilota*, used as a model species, in Hong Kong waters.

In Hong Kong, the global hub of the beche-de-mer trade, a dozen high-value species were identified in the local market. A hedonic analysis revealed that prices varied mainly according to species, size and physical damage attributable to inappropriate post-harvest processing. Higher prices were significantly associated with larger beche-de-mer, except for the temperate *Apostichopus japonicus*. As many beche-de-mer were in their sexually immature size-ranges, effective application of minimum size-limits regulations would benefit from economic incentives derived from species-specific relationships between size and price.

Reproduction in *Holothuria leucospilota* was determined in Hong Kong, which could be studied in a non-exploited state. The relationship between body size and gonad index was examined in detail. Risks associated with the misinterpretation of the gonad index are rarely considered in holothuroids research, so this work explored the problems that arise when different body sizes are sampled over time and an allometric relationship holds between gonad and body weights. An innovative gonad index was successfully applied, removing the confounding effect of body size, and describing the same reproductive cycle as histological gonad analysis.

To validate the use of fluorochromes to batch-mark *Holothuria leucospilota*, experiments were conducted in outdoor tanks. Preliminary calcein marking could be detected for a year in microscopic ossicles extracted non-lethally from dermal samples. Four fluorochromes, calcein, calcein blue, xylenol orange and tetracycline, successfully marked ossicles of juveniles and adults *H. leucospilota* for one year. Marked ossicles were detected after a year with 4% error in tag detection. Growth of holothuroids was not affected by fluorochrome marking, although consistent weight loss occurred under experimental conditions.

Several methods, from enclosure experiments and modal progression analysis to batch-tagging mark-recapture experiments, were investigated to determine growth of *H. leucospilota*. Although an effect of captivity was suspected, the variation in weight of *H. leucospilota* in the enclosure experiment appeared to be seasonal. Consistent negative growth rates during winter were confirmed.

Finally, as size is an important characteristic of the economic value of beche-de-mer, further investigation on growth, longevity and reproductive capacity is essential for assessing stock renewal and potential for aquaculture. Based on life-history strategies exhibited by *H. leucospilota* in Hong Kong, further recommendations were proposed for fisheries management of Indo-Pacific species in the Asian subtropical area.

Chemical communication in marine symbioses: Characterization of the kairomones in two crustacean-echinoderm association models

Guillaume Caulier (Biology of Marine Organisms and Biomimetics, 6 Av. Champ de Mars, University of Mons, 7000 Mons, Belgium. Email:guillaume.caulier@umons.ac.be)

Chemical sensing is considered as the most ancient and the most ubiquitous mode of communication in the biosphere; all living organisms are able to detect chemical cues (or ecomones) in their environment to establish intra or interspecific interactions. In marine ecosystems, chemical communication is particularly involved in the host selection and the maintain of symbiotic associations. However, marine chemical ecology is a recent science and there is a lack of knowledge about the identification of the secondary metabolites allowing symbionts recognition. The main aim of the thesis presented by Guillaume Caulier in October 2016 is to characterize the nature and the effects of the molecules (i.e. kairomones) detected by crustaceans to select their echinoderm hosts. Two obligatory symbiotic models were investigated: the Harlequin crab *Lissocarcinus orbicularis* (Dana, 1852) associated with different species of holothuroids and the snapping shrimp *Synalpheus stimpsoni* (De Man, 1888) associated with different species of crinoids.

The study is divided into five chapters, each corresponding to a particular question: i) What are the population characteristics of *L. orbicularis* and the holothuroids on the Great Reef of Toliara (Madagascar)? (Caulier et al.

2012); ii) What is the diet of the Harlequin crab? (Caulier et al. 2014); (iii and iv) Which chemicals are involved in host selection for *L. orbicularis* (Caulier et al. 2013) and *S. stimpsoni*?; v) Do symbionts and predators (i.e. *Thalamita crenata*) rely on the same kairomones to elicit host/prey selection? Do chemodetection behaviors change when the host/prey is diseased?

Evidences provided within this work allow us to better understand the symbiotic association between the Harlequin crab and holothuroids in terms of host selection and symbiont feeding strategy. Results show that holothuroid saponins and crinoid anthraquinones, initially described as chemical defenses, play the role of kairomones attracting the symbiotic crustaceans because they act as chemical signatures. Moreover, this work describes the "diagnosis ability" of predatory and parasitic crabs detecting holothuroids with skin ulceration disease thanks to their olfaction.

Microbial – deposit feeder aquaculture bioremediation systems

Georgina Robinson (School of Marine Science and Technology, Newcastle University, September 2016)

Land-based intensive aquaculture produces large volumes of particulate organic waste that can be upcycled into high value secondary biomass. In this research, the application of two key principles underpinning low-cost bioremediation technologies, namely the addition of rate limiting (i) electron acceptors (oxygen), and (ii) donors (carbon) is investigated in a sediment-based aquaculture effluent treatment system integrating the sea cucumber, *Holothuria scabra*. Growth trials of *H. scabra,* combined with next generation sequencing (NGS) technologies, were used to examine the response of sea cucumbers and sediment bacterial communities under contrasting redox regimes, describing fully oxic and redox-stratified sediments. The oxic system resulted in high taxonomic and functional diversity of bacteria with a range of dissimilatory metabolisms required for successful bioremediation of aquaculture wastes; however, the final biomass of *H. scabra* was significantly lower than the redox-stratified sediments (449.22 ± 14.24 g m^{-2} versus 626.89 ± 35.44 g m^{-2}). Improving the resource quality of aquaculture waste through carbon supplementation was investigated. Increasing the carbon/nitrogen ratio from 5:1 to 20:1 with soluble starch significantly increased the biomass production of *H. scabra* on redox-stratified sediments compared to controls (1011.46 ± 75.58 g m^{-2} versus 702.12 ± 35.93 g m^{-2}). A benthic flux incubation study, combined with NGS, demonstrated that carbon supplementation did not change the pathway of nitrogen cycling by mediating a shift from net release of ammonium to net assimilation, as hypothesised. A final study elucidated the critical role of the sea cucumber microbiome during aquaculture waste bioremediation, demonstrating that endogenous bacteria are primed, at ecological and genomic levels, to respond to nitrogen — a key nutrient limiting deposit feeder growth. Deposit feeder–microbial aquaculture bioremediation systems have the potential to rectify current inefficiencies of nitrogen use in the aquaculture production chain by offering a more economically and environmentally sustainable alternative to closing the nitrogen cycle loop.

Canaries in the coal mine: using model echinoderm species to address anthropogenic impacts on the Great Barrier Reef

Kennedy Wolfe (The University of Sydney, 2017)

Many beche-de-mer holothuroids are in a perilous state of conservation. *Stichopus herrmanni*, listed as Vulnerable by the International Union for Conservation of Nature (IUCN), is currently a major fisheries target on the Great Barrier Reef (GBR) and elsewhere. However, there remains a paucity of data on the biology and ecology of this species to inform management – an issue for bêche-de-mer holothuroids globally. This thesis bridges fundamental knowledge gaps for *S. herrmanni*, a model species used to represent other exploited tropical holothuroids, information critical to their conservation biology. The population biology, recruitment and ecological roles of *S. herrmanni* were examined across several years on protected zones on the southern GBR (Heron Island, One Tree Island). Population surveys showed distinct spatial variation in the size and density of *S. herrmanni* across Heron Island. The smallest individuals found (10 cm in length) are some of the only juvenile *S. herrmanni* documented in nature. The presence of juveniles in the consolidated crustose coralline algal habitat each year in autumn following the summer spawning period, and the absence of small individuals several months later in spring, suggests an ontogenetic migration or displacement of *S. herrmanni* to deeper lagoon areas as they grow. Across three years, *S. herrmanni* exhibited decreased feeding activity and gonad development in winter, with gonad development peaking in summer. Spawning observations indicate that gametes are released across new moon events during summer on the GBR. Adult *S. herrmanni* were calculated to turn over an estimated 100–250 kg ind^{-1} y^{-1}, the highest contribution to sediment bioturbation reported for an aspidochirotid. Sediment analyses suggest *S. herrmanni* is a generalist feeder that consumes benthic microalgae and infauna, influencing trophic interactions in its sediment habitat. Using lagoon sediment mesocosms, the potential for *S. herrmanni* to alter reef carbonate dynamics under present-day and near-future (+570 ppm) ocean acidification scenarios was examined. The ability for this species to alter benthic communities and modulate carbonate chemistry dynamics in lagoon environments suggests that large tropical holothuroids may serve an important role in mitigating the impacts of ocean acidification on coral reefs. This unappreciated feature of the feeding biology of tropical holothuroids indicates major concerns for reefs where bêche-de-mer species are exploited.