Cold-Water Coral in Aquaria: Advances and Challenges. A Focus on the Mediterranean

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Abstract

Knowledge on basic biological functions of organisms is essential to understand not only the role they play in the ecosystems but also to manage and protect their populations. The study of biological processes, such as growth, reproduction and physiology, which can be approached *in situ* or by collecting specimens and rearing them in aquaria, is particularly challenging for deep-sea organisms like cold-water corals. Field experimental work and monitoring of deep-sea populations is still a chimera. Only a handful of

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research institutes or companies has been able to install *in situ* marine observatories in the Mediterranean Sea or elsewhere, which facilitate a continuous monitoring of deep-sea ecosystems. Hence, today's best way to obtain basic biological information on these organisms is (1) working with collected samples and analysing them *post-mortem* and / or (2) cultivating corals in aquaria in order to monitor biological processes and investigate coral behaviour and physiological responses under different experimental treatments. The first challenging aspect is the collection process, which implies the use of oceanographic research vessels in most occasions since these organisms inhabit areas between ca. 150 m to more than 1000 m depth, and specific sampling gears. The next challenge is the maintenance of the animals on board (in situations where cruises may take weeks) and their transport to home laboratories. Maintenance in the home laboratories is also extremely challenging since special conditions and set-ups are needed to conduct experimental studies to obtain information on the biological processes of these animals. The complexity of the natural environment from which the corals were collected cannot be exactly replicated within the laboratory setting; a fact which has led some researchers to question the validity of work and conclusions drawn from such undertakings. It is evident that aquaria experiments cannot perfectly reflect the real environmental and trophic conditions where these organisms occur, but: (1) in most cases we do not have the possibility to obtain equivalent *in situ* information and (2) even with limitations, they produce relevant information about the biological limits of the species, which is especially valuable when considering potential future climate change scenarios. This chapter includes many contributions from different authors and is envisioned as both to be a practical "handbook" for conducting cold-water coral aquaria work, whilst at the same time offering an overview on the cold-water coral research conducted in Mediterranean laboratories equipped with aquaria infrastructure. Experiences from Atlantic and Pacific laboratories with extensive experience with cold-water coral work have also contributed to this chapter, as their procedures are valuable to any researcher interested in conducting experimental work with cold-water corals in aquaria. It was impossible to include contributions from all laboratories in the world currently working experimentally with cold-water corals in the laboratory, but at the conclusion of the chapter we attempt, to our best of our knowledge, to supply a list of several laboratories with operational cold-water coral aquaria facilities.

38.1 Aquaria Maintenance and Experimental Work with Cold-Water Corals. From the Beginning to the Present

38.1.1 The Challenges of Mimicking the Natural Environment

Conducting experimental work with cold-water corals (CWCs) in aquaria is a fairly recent research field. To our best knowledge, the first attempts to keep CWCs alive were conducted in 1980 when G.A.B. Shelton kept fragments of the reef-building CWC *Lophelia pertusa* in the Department of Zoology at the University of Oxford in order to study the behaviour of this coral (Shelton 1980). He was specifically interested in the electrical conduction mechanisms underlying coral behaviour and coordination. In the laboratory, *L. pertusa* has been successfully reared in the facilities of Tjärno Marine Lab (University of Gothenburg, Sweden) since the late 1990s.

Eighteen after the initial work by Shelton, Mortensen and Rapp (1998) studied the growth patterns of the same coral species, maintaining colonies in aquaria with running seawater for more than 18 months in the Trondhjem Biological Station (Norwegian University of Science and Technology).

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This pioneering work allowed obtaining the first *ex situ* data on this coral, contributing to increase our knowledge of a basic and fundamental aspect of the biology and ecology of a CWC species, spurring the modern era of experimental CWC studies. Starting in 1999, P. Mortensen further developed methods for studying the growth and behaviour of *L. pertusa* in aquaria (Mortensen 2001); this work demonstrated that it is possible to keep corals alive in closed water circulation systems for more than a month, and for more than a year with a continuous supply of seawater. Keeping *L. pertusa* in aquaria for such a long period allows for controlled experiments covering many aspects of its biology (e.g. behaviour, physiology, growth and reproduction).

After these pioneer studies, the field rapidly evolved in the last two decades. Large advances have been achieved in both the technical aspects regarding aquaria infrastructures to maintain CWCs alive (e.g. Olariaga et al. 2009), and the development of specific experimental set-ups to investigate particular aspects of the biology, physiology and ecology of CWCs. The first years of the twenty-first century were particularly fruitful in this respect. Dodds et al. (2007) published the first aquarium-based ecophysiology study on *L. pertusa*. Dodds and her team conducted experiments to study the respiratory physiology of *L. pertusa* under altered temperature and oxygen levels at the Scottish Association of Marine Science (SAMS), using aquaria infrastructures specially designed to maintain CWCs. The first results on growth rates of Mediterranean specimens of *L. pertusa* and *Madrepora oculata* measured in aquaria were published in 2008 (Orejas et al. 2008). That same year, Maier (2008) published the first work on the recovery capacity of *L. pertusa* in aquaria from tissue injuries. After these initial studies, several experimental works on feeding ecology of CWCs were conducted with different CWC species (e.g. Purser et al. 2010; Tsounis et al. 2010; Reynaud and Ferrier-Pagès, this volume; more details given in Sect. 38.5.2).

During the last 15 years, advances in the experimental approaches have increased exponentially. Several studies on growth rates in aquaria of CWCs from the Mediterranean and elsewhere have been published (e.g. Maier et al. 2009, 2012; Orejas et al. 2011; Naumann et al. 2011, 2013; Lartaud et al. 2013, 2014, this volume) as well as studies on the physiological response of CWCs to various experimental conditions (e.g. Gori et al. 2014a, 2015; Roik et al. 2015; Reynaud and Ferrier-Pagès, this volume). These studies included experiments conducted under future IPCC (Intergovernmental Panel on Climate Change) scenarios of global warming and acidification (Maier et al. 2009, 2012, 2013a, b, 2016, this volume; Movilla et al. 2014a, b, this volume; Carreiro-Silva et al. 2014; Hennige et al. 2015; Gori et al. 2016, among others), as well as experiments carried out to better understand the potential consequences of deep-sea drilling activities on CWC habitats (Larsson and

Purser 2011), or the effects of oil spills on these species (DeLeo et al. 2016). The advances in rearing *L. pertusa* has also lead to a number of successful spawning seasons in the laboratory when embryo development and larval behaviour could be studied (Larsson et al. 2014; Strömberg and Larsson 2017). The current chapter provides an overview of the advances made on the maintenance and *ex situ* experimenting with CWCs, placing the focus in the Mediterranean region, but also adding integrative relevant experiences from elsewhere.

38.2 Cold-Water Coral Sampling and Maintenance on Board

This section presents an overview of different approaches used for CWC sampling as well as maintenance on board and transport to home laboratories. Some experiences on short-term experiments on board are also included.

38.2.1 Cold-Water Coral Sampling and Transport to Home Laboratories

Over recent years several methods have been employed for the collection of CWCs for maintenance and experimentation, such as dredges, box-cores, grabs, remotely operated vehicles (ROV) and manned submersibles (e.g. Mortensen 2001; Roberts et al. 2006; Brooke et al. 2009; Maier et al. 2009; Orejas et al. 2011; Taviani et al. 2011), as well as through opportunistic sampling of corals accidentally captured (as bycatch) in fishing vessels (e.g. Sampaio et al. 2012). The collection of living CWC could be a relatively "simple" operation, but the subsequent procedure of keeping them alive on-board of the ship and their later safely transfer to a designated scientific aquarium can be a highly demanding task and needs appropriate preparation. It is generally acknowledged that more impacting methods such as dredges can cause significant stress to the organisms (visible as increased mucus production, or polyp retraction for extended periods of time), and can cause severe damage to coral tissue. Indeed, any method to collect living CWC from their habitats unavoidably generates a profound stress on the coral polyps. Entire coral colonies or coral fragments are suddenly and dramatically extracted from their habitat, reposed in canisters or analogue containers on the ROV or submersible, and exposed to changes in pressure and thermal shocks during their journey from the seabed to the surface. The induced stress represents a significant loss of energy, and can affect the survival of corals in captivity. Here we provide basic and practical information on how to best maintain coral viability, largely derived from empirical experience (Box 38.1).

Box 38.1: Best Practice for Ship-Board Maintenance of CWC

Immediately after collection, corals should be transferred with care to temperature-controlled aquaria filled with deep-sea water (collected with Niskin bottles from a CTD-Rosette or with a submersible water pump, for instance). Coral maintenance on board may be pursued through sophisticated or less sophisticated systems, according to the situation. Whenever possible, the best practice is to set-up a laboratory equipped with plastic or glass containers filled with seawater maintained at deep-sea temperature. Ideally, a closed system benefits survival chances. Flow is maintained by recycling filtered seawater between the container and a chilling unit connected to a pump, with an aeration system. Handling should be minimised as much as possible during the following weeks to allow corals to acclimate to aquarium conditions. Gently washing the corals with a small water stream from the tank, could help to keep corals clean. Further this also helps to eliminate the mucus layer they generate after the stress of the capture, increasing remarkably the survival rate. Corals should not be fed during this initial period as they will generally not eat (polyps are frequently retracted during the first days) and food decomposition may negatively affect water quality. From our experience, clean water and high current flow are more relevant for maintaining coral viability than abundant food.

To ensure maximal survival rates and the best physiological condition, corals should be preferentially collected with video-assisted technology (ROV, manned submersible) to minimise damage during collection. After the experience from M. Carreiro-Silva and her team from IMAR (Azores), the use of a thermo-insulated bio-box installed in the ROV with close-fitting lid is particularly recommended for corals collected at greater depths. The bio-box allows the storage of corals with deep-sea water, minimising exposure to temperature fluctuations during ascendance to the surface. To further minimise the risk of mortality, it is fundamental that coral colonies are quickly transferred from the stressing deck conditions to an environment that mimics as much as possible their original ambient situation; in practice this is primarily accomplished by placing the corals in a cold seawater tank in darkness. On the open ocean seabed, CWCs typically occur in environments with temperatures ranging from 4 to 8 °C (although summer temperature can reach 12 °C off Norway or in the Gulf of Mexico), depending on location. In the deep Mediterranean Sea, temperature is about 12 °C with little deviation from this figure (e.g. maximal temperatures of

14 °C have been registered in the Ionian Sea and of 13 °C in the Gulf of Lions). The geographic location of the Mediterranean basin translates into considerably high surface temperatures, especially in summer months, typically the season when many oceanographic surveys are conducted. These issues must be taken into account when planning the ship-board maintenance and later transfer of live CWCs.

Minimising coral air exposure after collection is another important factor, particularly when collecting gorgonians, because some species oxidise (become black) when exposed to air and die rapidly (e.g. *Dentomuricea* aff. *meteor*, *Acanthogorgia* spp.).

Even for less sophisticated systems, some good planning is advisable. The maintenance of corals after collection may also be achieved either by temporarily storing them in on-board refrigerators or cold rooms at temperatures between 10–12.5 °C for Mediterranean CWCs. As mentioned, seawater for on board maintenance should be collected in advance, giving preference to ambient bottom seawater, rather than surface water. This will provide seawater at ambient temperature and avoid potential contamination with microorganisms from shallower depths untypical for CWC sites. Water collected by means of Niskin bottles can also be stored in a dark cool room for some days to replace the water from the tanks if necessary. This is recommended if weather conditions or stays at harbours do not allow collection of seawater on a regular basis.

Under situations where ideal storage devices and equipment are unavailable (tank, seawater flow, aeration system), the collection and maintenance of living corals can be best safeguarded by keeping corals in a bucket filled with cooled seawater, ensuring frequent water substitution to provide oxygenation and storing if possible in darkness until transport and transfer to the selected aquarium can be arranged.

The aquarists from the Aquarium Finisterrae (A Coruña, Spain), who frequently collect deep-sea organisms (including corals) for exhibition and experimental purposes, use a 300 L tank to keep animals alive, if space is available on board the collection vessel. They place coral fragments in plastic grid boxes inside the tank to avoid samples moving out of place and getting hit during transportation. The tank is covered with a lid and kept under constant water inflow (open circuit) until it arrives to land. The specimens from deep areas (800–1200 m depth) are immediately put into an isothermal 400 L tank with a watertight cover and equipped with a chiller unit that maintains water temperature at a level similar to their natural habitat. This tank also has mechanical, biological and chemical filtration as well as a protein fractionator, an ultraviolet (UV) unit and a recirculating pump that generates water currents inside the tank (Fig. 38.1). This system is very convenient for expeditions that take several days, as it allows holding CWC in a closed circuit for 10–15 days.

Fig. 38.1 The isothermal tank used by the Aquarium Finisterrae to keep deep-sea fauna alive at sea. The tank is located on the top of the so called "vital support" system which contains a skimmer and mechanical, chemical and biological filters (all of them placed in the black box). Behind the isothermal tank (not visible in the photograph) are located the ultraviolet unit as well as the chiller. (Photo: © A. Veiga)

The following steps concern sample transportation to the scientific aquaria facilities and coral transfer and acclimation into the aquaria. These operations are also highly critical for the viability of corals. Corals need to be transferred from the ship to a vehicle while keeping them refrigerated. A fully equipped cooled vehicle is the best solution for such purpose. Alternatively, electric or cool boxes, portable refrigerators and/or thermal bags may be adequate. Careful planning of appropriate paperwork may also be required. It is necessary to be aware of the protection status of many CWC species, as for instance all scleractinian and antipatharian corals are included in CITES Appendix II (https://www.cites.org) and many are protected under different regulations (in the case of the Mediterranean Sea, it is necessary to check the Barcelona Convention: http://web.unep.org/unepmap/).

Another important aspect to consider is to ensure that the requirements set forth by port authorities are met in order to get the official clearance to leave the harbour. Living corals may also be transported by other means than land vehicles, such as ships or aeroplanes. It should however be kept in mind that not all air companies allow the transport of living corals.

The final destination for collected corals is often a scientific laboratory or exhibition aquarium. These host aquaria

should be aware of the type of material to be received and the time of arrival. It is the responsibility of the host aquaria to adjust the system settings in advance (aerators, filters, proper seawater temperature, and light level) to guarantee the wellness of the corals. A few days of acclimation are optimal to progressively reduce the level of stress. To achieve acclimation it is important to monitor polyp activity and tissue condition several times per day. Stress signs include closed polyps, and tissue sloughing and loss, as well as extensive mucus production. Assessing tissue condition is relatively easy in corals with coloured tissue, as it is the case for the yellow *Dendrophyllia cornigera*, but in the case of the white corals, the degree of polyp opening should be used as a "control" of coral condition instead. Different methodologies can be applied to help in reducing the signs of stress and achieve acclimation, depending on which are the feasible logistics. If ambient seawater is available, a slow mix of this water and the water of the home aquaria is a good strategy to facilitate coral acclimation. Current speed should also be tested and optimised to achieve polyp extension, as this is frequently one of the most important aspects for successfully keeping corals in a good shape. From our experience, feeding should be restricted during the first days following transfer, however after a couple of days, the addition of liquid food close to the polyps can stimulate their extension, probably as a result of stimulus on coral's chemical receptors. Once the polyps are open it is also easier to check which is the current speed they prefer. In the specific case of Mediterranean CWCs collected for tank experiments, various *Lophelia pertusa* and *D. cornigera* collected in 2006 and 2008 are still alive and in healthy conditions in the aquaria facilities of the ICM-CSIC (Barcelona, Spain) and the CSM (Monaco) respectively.

38.2.2 Short Term Experiments on Board

Fully equipped research vessels may provide opportunities to conduct short-term experiments with live CWCs on board (e.g. Maier et al. 2006, 2007; Taviani et al. 2011; Hennige et al. 2014; Orejas et al. 2017). Such practice presents advantages and disadvantages. Advantages include: (1) measuring physiological functions very shortly after sampling using freshly collected specimens that might be more closely mimicking the response of *in situ* conditions than specimens maintained over long periods under more artificial aquarium conditions; (2) the possibility to use seawater from the coral collection point, instead of artificial seawater or water from shallow areas. Indeed, obtaining clean seawater can be expensive and time consuming, and coastal water does not always mimic conditions found near CWC habitats. This is particularly true if corals are maintained in a region different from where they were collected; for example, the chemistry of the Pacific and Atlantic oceans differs greatly. Disadvantages

include: (1) short acclimation time even in long cruises; (2) the response of the organisms is only "short term" and need to be considered with caution. Some species are tolerant to sub-optimal conditions, thus experiments conducted under these conditions should be interpreted with caution. This is especially the case if corals have been living in aquaria for extended periods (e.g. years), since its unknown if aquaria conditions can modify basic aspects of the physiology of CWCs, related to changes in feeding regimes and chemical properties of seawater. Therefore both short- and long-term experiments encompass limitations with respect to the interpretation of a CWC response to measured variables for different reasons which should be kept in mind when evaluating *ex situ* experiments in general.

In the following paragraphs some selected examples of short-term experiments conducted on board research vessels are presented.

In 2012, S. Hennige from the University of Edinburgh and his co-workers conducted short-term ocean acidification (OA) experiments under different temperature regimes, on freshly collected *L. pertusa* samples on board of the British RRS *Discovery* (Hennige et al. 2014). Corals were maintained on board in experimental 'coral hotels', which are self-contained 430 L units containing ~350 L of seawater, with built in water circulation pumps, filtration units and chillers (Fig. 38.2).

Water circulation in these closed systems was ~300 L h^{-1}. To alter the carbonate chemistry in the experimental tank, pre-mixed (e.g. with mass flow controllers) or purchased gases with elevated CO_2 were bubbled continuously into the water. To reduce the change in pH following water changes, freshly collected seawater was pre-bubbled prior to addition to the tanks. Subsequently, 30% of the seawater in the tanks was exchanged every 2 days following feeding to ensure build-up of detritus to be minimal. This kind of experimental set-up contributed to get insight on the short-term behaviour and physiological response of CWC to the predicted effects of global climate change (global warming and OA). Results of these experiments are reported in Hennige et al. (2014, 2015).

Another example of short-term experiment on board is a study conducted by A. Gori and C. Orejas (unpublished data) in 2015 during the ANNA cruise on board the German RV *Meteor* off Angola, a location where the CWC *L. pertusa* builds impressive reefs under very low oxygen (O_2) concentrations. They conducted a physiological experiment on board aimed at analysing the physiological response under the natural low O_2 conditions (2 mL L^{-1}) and saturated O_2 conditions by measuring respiration, ammonium excretion and calcification rates of the corals. The experimental set-up on board consisted of incubation chambers (~400 mL volume), which were filled with water collected close to the seafloor using Niskin bottles. Each experimental chamber included a stirrer to keep the water in movement; once filled, chambers were closed with a lid and sealed with parafilm to avoid any gas exchange. During the entire experiment water renewal was manually carried out every 6 h in order to provide the corals with fresh seawater and to avoid an ammonium increase in the chambers. Temperature and O_2 concentration were carefully controlled in every chamber before each water renewal. Figure 38.3 displays the experimental set-up on board of the RV *Meteor*. No differences were observed in the respiration rates between corals maintained under natural low O_2 and increased O_2 conditions, whereas respiration significantly increased during the week of incubation.

From 2005–2008 cruises to the North Atlantic and Skagerrak took place on the Dutch RV *Pelagia* equipped with a cool- and a radioisotope container for on board experimentation with CWCs and deep-sea sponges (van Duyl and Duineveld 2005; Maier et al. 2006, 2007). This allowed the study of nutrient dynamics and the role of prokaryotes on CWC ecosystems using freshly collected specimen of *L. per-*

Fig. 38.2 "Coral hotels" used at SAMS to keep the corals alive. The compartment in the right contains the chiller and electronic devices and the compartment in the left is filled with sea water to host the corals. (Photo: © A. Gori)
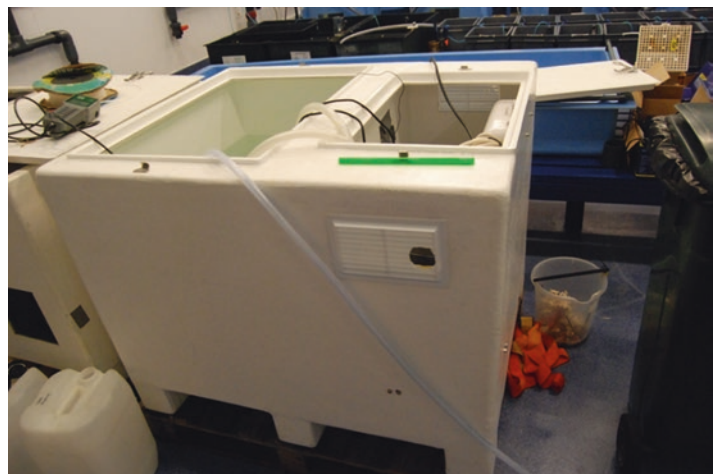
Fig. 38.3 Maintenance and experimental set-up for CWC on board RV *Meteor*. **a**) Two chillers are connected to two tanks (acclimation and experimental) to maintain a constant temperature, **b**) experimental set-up with control and experimental jars (containing *Lophelia pertusa* nubbins) to measure respiration and calcification rates. The water is kept in motion by the shaking plate underneath the jars and the stirrers inside the jars. (Photos: © A. Gori)
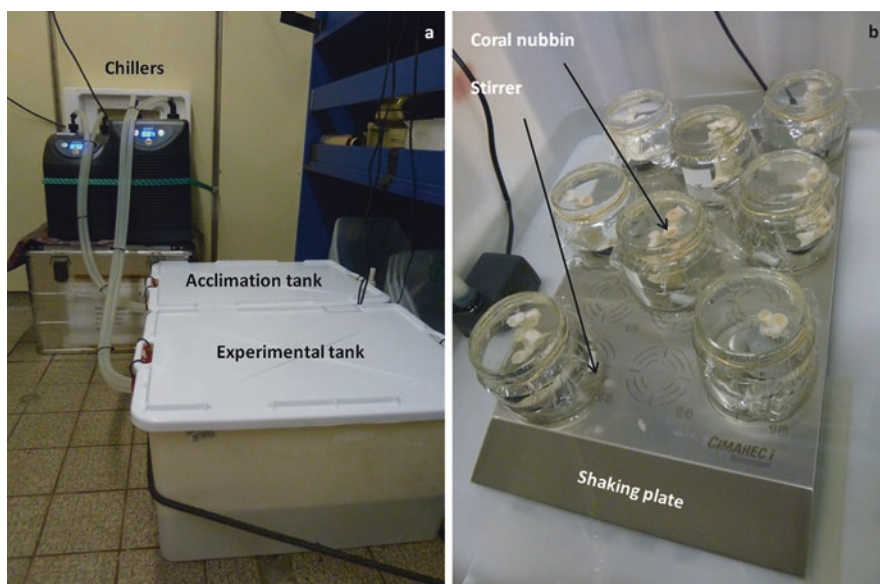

Fig. 38.4 Sampling of CWC during cruises in the North Atlantic and Skagerrak. (**a**) Sampling of CWCs using a box corer with a lid. The box corer was sealed with bottom substrate avoiding contamination of bottom seawater with shallower water when hauling the box core back on board, (**b**) ambient bottom water used for on board experiments was sampled using a 1000 L water box, (**c**) *Lophelia pertusa* nubbins mounted in methacrylat bases to conduct the experiments on board. (Photos: © C. Maier)

tusa and *Madrepora oculata* (Fig. 38.4; Maier et al. 2011, unpublished data; Weinbauer et al. 2012) or sponges (van Duyl et al. 2008). During the cruises in 2007 and 2008, additional radiotracer studies were conducted using 45-calcium to measure skeletal growth of *L. pertusa* from two sites (Mingulay and Skagerrak) and to conduct a first test on the effects of OA on CWCs (Maier et al. 2009). As the calcification rates obtained during these first onboard experiments appeared realistic (unfortunately no comparative *in situ* data were available at that time, but see new recent data in Lartaud et al., this volume and references therein), the approach of on board experimentation was continued during two projects (MECCA and COMP) to study the calcification of Mediterranean CWCs under the lead of the Marine Protected Areas Agency (AAMP) on board the French RV *Minibex* (COMEX), as well as during the MEDCOR cruise 2009 on board the Italian RV *Urania* (Maier et al. 2012). Currently

there are published *in situ* growth rates measurements for *L. pertusa* and *M. oculata* from the Mediterranean (Lartaud et al. 2013, 2014, 2017a, b, this volume) as well as for *L. pertusa* from the Gulf of Mexico (Brooke and Young 2009). Results indicate comparable growth rates for *in situ* and *ex situ* measurements for old polyps of both species and faster growth rates for young polyps of *L. pertusa* in the Mediterranean (Lartaud et al. 2013). In contrast, for *L. pertusa* from the Gulf of Mexico, growth rates comparable to those in the lower range obtained in aquaria, have been documented (Brooke and Young 2009).

To minimise stress during CWC collection, a special sampling device was designed by the COMEX engineers. This device is a miniature copy of the "Croix St. André" formerly used to sample the precious red coral *Corallium rubrum* (Fig. 38.5a, b). The device is an extremely efficient and less destructive sampling tool reducing fragmentation of coral
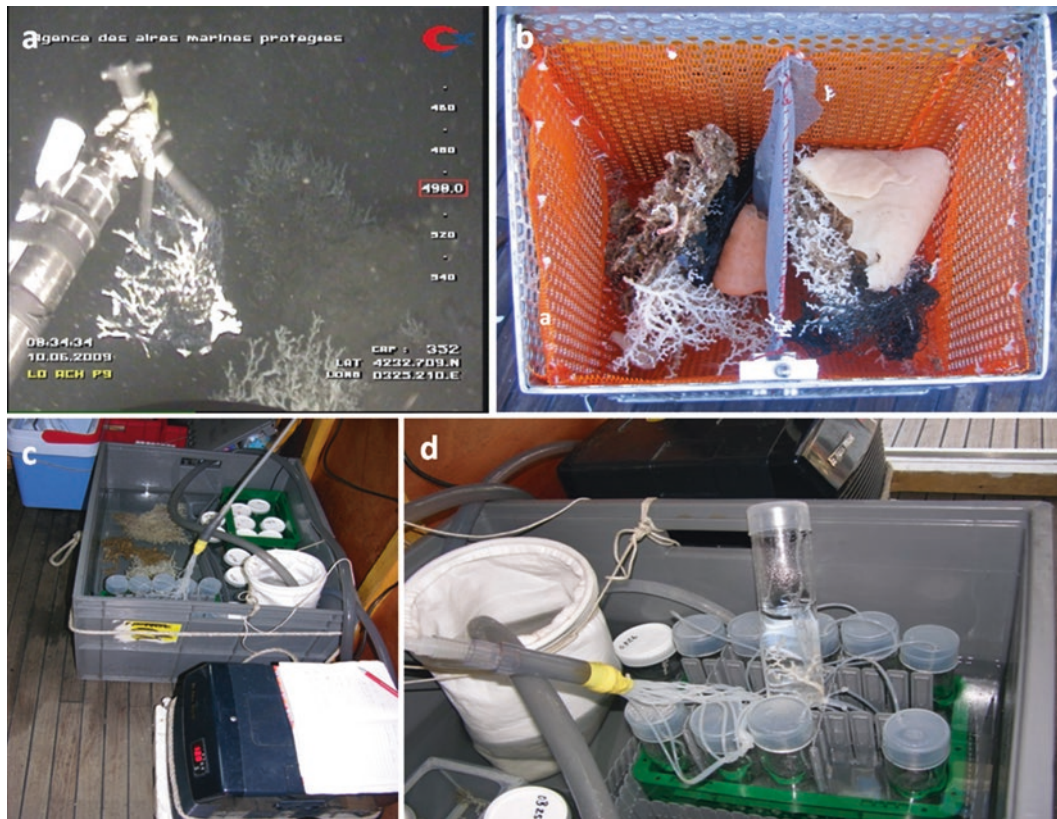
Fig. 38.5 Sampling and on board experiments during Minibex cruises in the Mediterranean. **a**) The ancient sampling gear "Croix St. André" was the inspiration to construct a miniature version to more efficiently sample small CWC colonies with the ROV that was dropped together with the colony entangled in the mesh ends into the buckets on the ROV (**b**). On board maintenance and experiments were conducted in a single container equipped with chilling unit, circulation pumps and a filtration unit consisting of two micron bags with 5 and 1 μm mesh size (**c**, **d**). (Photos: © C. Maier)

branches that may otherwise lost in the soft sediment underneath. On board, the experimental set-up was kept simple using either open (aerated) or closed incubation containers that were kept in the CWC storage container at 13 °C. In closed system incubations, the seawater chemistry can change significantly as a result of coral metabolism, chamber volume and time of incubation (Maier et al. 2009, 2012). Despite the changes in carbonate chemistry, calcification rates derived from closed system incubations proved to be comparable to open system incubations (Maier et al. 2012) and were comparable to those obtained in long-term aquarium experiments (Maier et al. 2013b) and *in situ* measurements (Lartaud et al. 2014, 2017a, b). Closed system incubations are easier (and frequently the only ones) to use on board and might therefore facilitate large scale interregional comparisons of CWC calcification growing under different environmental conditions with respect to temperature, salinity and carbonate chemistry. Another advantage of closed system incubations is that both calcification and respiration can be determined in parallel and together with the determination of other parameters (dissolved inorganic carbon, total organic carbon) the CWC carbon (C) and energy budget can be determined from a single incubation (Maier et al. 2016).

38.3 Cold-Water Coral Aquaria Facilities in Research Institutes, Universities and Public Aquaria

This section intends to offer an overview of some of the aquaria facilities currently available in different research institutes, mostly in European laboratories but also from the USA. Even though this book focuses on Mediterranean CWCs, we considered that in such a recent field of research as the maintenance and experimental work with CWCs, any experience in the husbandry of CWCs will be of great use to all researchers (Box 38.2). The facilities of 9 research institutions are presented here as examples of possible approaches which may be employed, and at the end of the chapter we include a list with some of the aquaria infrastructures for CWCs we have been able to identify. We hope we will be able to update this list in future editions of this volume.

Box 38.2: Best Practices on Basic System Requirements

The choice of the most adequate aquaria system to keep CWCs depends on the proximity to the sea, and the amount of money available for system set-up and maintenance. The best aquarium design is an open water system with a continuous intake of seawater. Ideally, seawater should come from the deep to minimise differences in water quality parameters between the aquaria and natural habitat. However, setting up a deep-sea water intake is expensive, and if salinity of a shallow water source does not vary a lot, this is also a good alternative. Having a constant water supply has the advantage of easy removal of waste, avoiding the build-up of toxic nutrient levels, and continuous replenishment of oxygen and consumed minerals (e.g. calcium). Closed system can be efficient too, but are more expensive and technologically complex, requiring an elaborate filtration system.

– Aquaria systems should ideally be mounted inside a temperature controlled room to avoid large temperature variations in the aquaria. Aquaria systems may vary depending on their use (i.e. exhibition or experimentation) but should include stock / quarantine aquaria of at least 50 L capacity in addition to the systems for the actual experiments / research.

– Aquaria and their shelves should be as versatile as possible so this will not limit future experimental designs. There are several options for the aquaria building materials, depending on the budget, the purpose, durability and on maintenance requirements. For example, glass is stronger and cheaper, but also breakable and heavier than plexiglas (acrylic) or polycarbonates, but on the other hand some of these plastics might get slightly opaque or easily scratched with time.

– Seawater refrigeration units should be slightly over dimensioned, considering expected maximum water renewal / turnover / in flow rate, and they should be independent to each separate aquaria system mitigating possible damage / losses from one of the units breaking down.

– For closed aquaria systems the main focus of a basic filtration system is on the water quality in terms of nutrients / organic waste, and similarly to most common seawater aquariums, it should cover first of all the biological filtration, but also mechanical and chemical filtration.

– Biological filtration converts the waste products to a less to non-toxic state. This is mainly attained by various types of bio-filter systems and materials that maximise the growth potential of the naturally occurring nitrifying bacteria. The type or size should be according to the expected maximum bio-load and waste.

– Mechanical filtration serves mainly to collect particles that may be found in suspension in the water (organic and / or inorganic), protecting gears and parts from physical damage or clogging (e.g. sand getting into pumps) and collecting organic matter before it decomposes. But since we are working with filter feeding organisms, this also interferes with / compromises the availability of food.

– Chemical filtration, comprises not only the non-biological removal of dissolved substances from the aquarium (such as using activated carbon), but also other methods such as foam fractioning (or protein skimming), ozonation and ultraviolet sterilisation. Although none of these is absolutely necessary, all of them can be very helpful in the export or mitigation of unwanted inorganic / organic material from the aquaria.

– For open system aquaria the water quality is mostly ensured by a proper water renewal (new water inflow). So, assuming a source of good water quality, the filtration system main focus should be on eliminating or mitigating possible inflow of harmful elements. This mainly includes mechanical filtration of inorganic suspended sediments, and / or organic matter or unwanted and possibly harmful living organism that may be coming along with the new water. Another approach to mitigate the possible inlet of living organisms may be chemical filtration, such as ozonation or ultraviolet sterilisation of the incoming water, thus disabling or even killing them.

– Evaluation of the ideal seawater renewal rate or in flow rate depends on the type of system (open or closed), type of filtration system, and number of corals in relation to volume of water in each aquarium. All these factors will influence nutrients and waste build-up and thus water quality in aquaria. Generally it can be considered good practice to replace 10–50% of seawater weekly or monthly from each aquarium using properly matured bio-filters.

– The type of submersible recirculation pumps (stream or pulsating), direction (laminar or turbulent) and current velocity depend on the coral being maintained (see Sect. 38.4)

– The control / monitoring of parameters usually comprise inorganic nutrients (e.g. ammonia, nitrite, nitrate and phosphates), pH, temperature, salinity

and total alkalinity (especially for closed systems). Depending on the experimental design, and objectives of the study, some other parameters might require monitoring.

– Lights should be kept off whenever possible, recreating the coral's natural environmental conditions. The use of red filters on the lights (or red light bulbs) may help reduce possible impacts by the necessary light exposure. In addition, this also helps to avoid unwanted algae proliferations in the aquaria.

– Electrical installation / wiring and plugs should be above water level with some safety distance to minimise the chances of getting water splashed and waterproof parts should be installed whenever possible. Extra plugs should be available, for lab equipment. Each aquaria system should have individual sets of electrical wiring / switches to ensure that an electrical problem on one system does not compromise the others.

38.3.1 Aquaria Facilities for Cold-Water Coral Maintenance at the Benthic Ecogeochemistry Laboratory (LECOB) of Banyuls-sur-Mer, France

The LECOB laboratory at Banyuls-sur-mer marine station (Oceanographical Observatory, Pierre and Marie Curie University) has been equipped with aquaria facilities for cold-water scleractinian and gorgonian corals since 2010. The laboratory is located on the West coast of the Gulf of Lions, in the Mediterranean Sea. This area is close to the Lacaze-Duthiers submarine canyon (canyon head is 23 km off Banyuls-sur-Mer), where *L. pertusa* and *M. oculata* frameworks occur, together with *Desmophyllum dianthus* and *Dendrophyllia cornigera* corals. This canyon and the coral ecosystems are part of the Gulf of Lions Marine Nature Park MPA.

The aquaria facilities include several tanks (Fig. 38.6) installed in thermo-regulated rooms allowing mimicking deep-sea temperatures. The cool-rooms are supplied with natural seawater pumped from 10 m depth. Once the water is transported into the cool-room, a storage tank is filled and temperature is regulated to reach the 13 °C by means of a chiller before filtration by a 5 μm mesh size filtered and after this distributed to the different aquaria. Water is continuously distributed to allow a complete water renewal from 1 to 4 times per day in each tank over 80 L tanks or 50 L recirculating flumes (Purser et al. 2010), depending on the experimental requirements. Corals were maintained in the dark, fed three times a week with live *Artemia* nauplii, and the environmental conditions (temperature, pH, conductivity, oxygen saturation) are continuously monitored. Safety equipment is installed to prevent any problems (water-level sensor, seawater cut-off, temperature anomalies, etc.). This experimental design was developed for medium (months) to long-term (years) experiments.

The experiments conducted in the Banyuls-sur-Mer infrastructures address questions regarding CWC response to environmental changes and anthropogenic threats, the first one in order to forecast the response of Mediterranean CWCs to current and future environmental conditions predicted by the IPCC. Integrative studies are executed at distinct physiological levels including skeleton growth, energy acquisition and coral associated bacterial communities (Lartaud et al. 2013, 2014; Meistertzheim et al. 2016). Conducted experiments aimed at investigate the resilience of reef-building CWCs and their function in canyon ecosystems, particularly regarding the effects of potential changes in temperature, current speed, and organic matter concentrations.

38.3.2 Cold-Water Coral Maintenance Facilities at the Centre Scientifique de Monaco (CSM) (Principality of Monaco)

The CSM has 10 years' experience in maintaining CWC species; specifically, *L. pertusa, M. oculata, D. cornigera, Dendrophyllia ramea* and *D. dianthus* sampled in deep waters
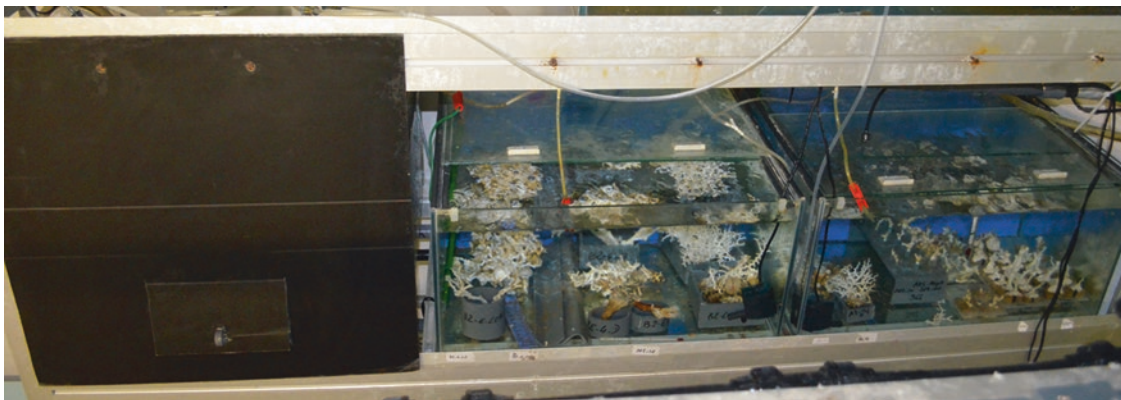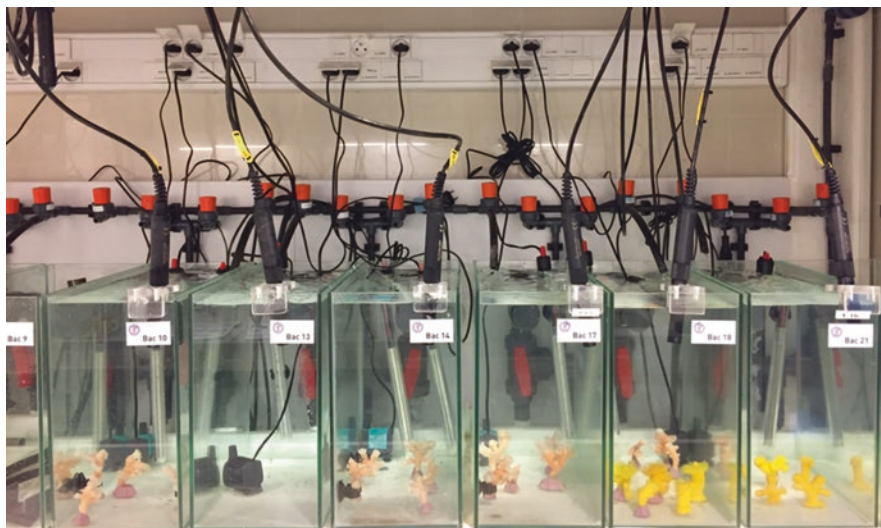

Fig. 38.6 Aquaria facilities in the Marine Laboratory of Banyuls-sur-Mer, France. (Photo: © E. Peru, LECOB)

Fig. 38.7 Coral fragments of *Dendrophyllia ramea* (pink/orange coral) and *Dendrophyllia cornigera* (yellow coral) in the small 30 L aquaria in the aquaria facilities of the CSM. (Photo: © S. Reynaud)

of the Mediterranean Sea, have been maintained in the CSM aquaria infrastructures. Currently, specimens of *D. cornigera* and *D. ramea* have been grown in the CSM aquaria for more than 6 and 2 years, respectively (Fig. 38.7). The corals are maintained in the dark, either in large 100 L aquaria for general maintenance (at 12 °C), or in 30 L aquaria for experimental purposes (at different temperatures). Seawater renewal is provided by a continuous flow of Mediterranean seawater pumped from 50 m depth at a rate of 1 m³ h⁻¹ and pre-cooled at 11 °C. Water temperature is then slightly heated to 12 °C or more using 300 W heaters connected to temperature controllers, or cooled down to lower temperatures using chillers. Submersible pumps with a flow rate of 400 L h⁻¹ provide continuous water movement inside each aquarium. Corals are fed 4 times a week with frozen *Mysis* (Crustacea, Eumalacostraca).

38.3.3 Cold-Water Coral Maintenance Facilities at the Institut de Ciències del Mar (ICM-CSIC) (Barcelona, Spain)

Cold-water corals have been maintained at Institut de Ciències del Mar (ICM-CSIC) since 2006 in an updated version of the system developed by Olariaga et al. (2009). The aquaria are located in a 15 m² temperature-controlled experimental chamber in the "Area of Experimental Aquaria" (ZAE) of the ICM-CSIC. Air temperature inside the chamber is maintained between 12 °C and 14 °C in complete darkness conditions. Corals are kept in four 140 L maintenance aquaria (Fig. 38.8) and twenty-four 25 L experimental aquaria. Seawater is provided by a continuous flow of Mediterranean seawater directly collected in front of the institute facilities and pumped from a depth of 15 m at a maximal inflow capacity of 300 L h⁻¹, then filtered with a 50 μm pore size mesh and cooled at 12 °C. Seawater enters the chamber directly to a 120 L storage tank, from where it is

pumped to the 28 aquaria. Continuous water movement inside each aquarium is provided by submersible pumps with a flow rate of 10,000 L h⁻¹ in the large maintenance aquaria and 2000 L h⁻¹ in the small experimental aquaria. Moreover, in the large maintenance aquaria, flow intensity can be regulated and wave action simulated. As a security measure, in case of malfunctioning of the seawater cooling system, the pumping of water from the storage tank is automatically blocked when seawater temperature exceeds 15 °C. In that case, corals are maintained at 12–14 °C in their aquaria by the air temperature controlled chamber. Currently, the aquaria harbour scleractinian CWCs, as well as gorgonians, black and soft corals and sea pens.

38.3.4 The "DeepSeaLab", Instituto do Mar (IMAR), University of the Azores in Faial Island (Azores, Portugal)

The DeepSeaLab at IMAR, University of the Azores on Faial Island is an aquaria facility dedicated to research with deep-sea organisms, conceived and designed in collaboration with the Oceanário de Lisboa in 2009 and subsequently renewed and enlarged in 2015. The aquaria facility is composed of 5 independent aquaria systems set-up in a temperature-controlled room at 14 °C in darkness. One semi-closed water system is used for the maintenance of corals and other deep-sea organisms that are not being used in experiments, and four open-water systems used for the experimentation (Fig. 38.9). The existence of a semi-closed water system ensures that in the case of seawater supply failure, corals can still be maintained in a fully closed system. All systems are equipped with a water temperature controller. The temperature sensors have 1 °C accuracy and a minimal hysteresis of 1 °C. The water supply to the aquarium systems is continuously pumped from coastal waters (5 m depth, salinity: 36).

Fig. 38.8 Overview of the CWC aquaria facilities at ICM-CSIC in Barcelona. (Photo: © S. Ambroso)

Fig. 38.9 (**a**) Aquaria infrastructures in the DeepSeaLab, (**b**) detail of a tank showing the water bath to minimise fluctuations in seawater temperature between replicate experimental aquaria. (Photos: © R. Sá da Bandeira)
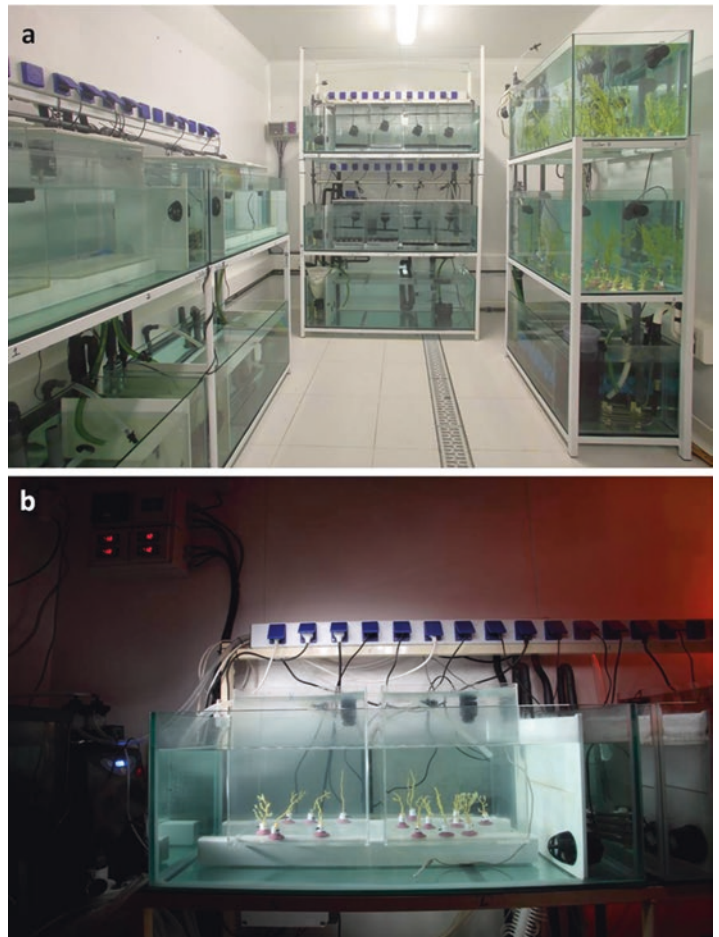
The water is stored in a 410 L storage tank equipped with a cooling unit and a thermostat. Water is filtered first through a 50 um felt filter bag (FSI®) in the storage tank and through an additional 5 um felt filter bag in each experimental system as well as UV sterilised. Water is then pumped into sumps in the individual systems where it is cooled again and subsequently distributed to the individual experimental aquaria. A freshwater water bath equipped with a cooling unit ensures that the water in individual experimental aquaria (e.g. replicate aquaria within the same experimental treatment) is constantly maintained, while maximising space for the experiments. Each aquaria system has the capacity to independently manipulate temperature and pH (through a combination of CO_2 bubbling and/or CO_2 removal through a soda lime filter) enabling the simulation of conditions predicted as consequences of climate change (Carreiro-Silva et al. 2014). In addition, the facility is equipped with 100 L stock tanks and dosing pumps for the delivery of sediments or food particles in different concentrations for feeding and deep-sea mining experiments. Currently, the aquaria maintain cold-water scleractinians, gorgonians, black corals, zoanthids and anemones.

38.3.5 Cold-Water Coral Aquaria Facilities of the "Changing Oceans Group" at the University of Edinburgh (Edinburgh, United Kingdom)

The Changing Oceans Group experimental mesocosm facility was rehoused and refurbished in 2017 to increase capacity and flexibility for conducting CWC and OA experiments, with flexible racking and tank systems. Seawater can either be collected from the East coast of Scotland, or artificially made.

An example set-up used previously (Fig. 38.10) had 20 independent closed-loop systems, each with an additional 4 experimental tanks (5 L each) for separation of biological material. Total volume of each system including the sump is 80 L, and includes individual temperature control and biological filtration. Gas mixing for OA experiments is achieved in house using mixing flasks, and analysed continuously with a Li-820 gas analyser (Licor). Ambient and elevated CO_2 air mixes (e.g. at 750 and 1000 ppm) are plumbed around the room and into sump tanks to modify water carbonate chemistry (Hennige et al. 2015; Gori et al. 2016).

38.3.6 Aquaria Facilities of the University of Gothenburg, Sweden (Sven Lovén Centre for Marine Infrastructure)

The marine station of the University of Gothenburg (UGOT) at Tjärnö offers excellent opportunities for both experimental and field based research on *L. pertusa*. The station is situated on the Swedish west coast in the vicinity (~10 nautical miles) of several shallow coastal *L. pertusa* reefs in the NE Skagerrak. The Tisler reef is the largest of these reefs with living coral extending over an area of ca. 250 ha at a depth of 70–145 m (Lundälv 2003). The facilities include several constant temperature rooms with a flow-through system with seawater taken from 45 m depth in the Koster Fjord, adjacent to the laboratory (Fig. 38.11). The fjord and surrounding areas are fed with deep oceanic water from the Atlantic that is funnelled via the Norwegian Trench allowing for simulation of deep-sea conditions in the laboratory. *L. pertusa* has been successfully reared in these facilities since mid-2000's (see Sect. 38.1).

Fig. 38.10 A previous set-up of the Changing Oceans Group experimental mesocosm facility. (Photo: © S. Hennige)

Fig. 38.11 CWC aquaria facilities at the marine station of the University of Gothenburg, Tjärnö, Sweden. (Photo: © C. Orejas)

38.3.7 Cold-Water Corals Aquaria Facilities at Temple University (Philadelphia, USA)

The aquarium system at Temple University in Philadelphia was first established in 2009 using primarily hobbyist equipment within a temperature-controlled room. In 2010, a second system was added within a standard laboratory room without temperature control, but including a temperature-insulated "lobster tank" paired with an aquaculture-grade recirculating chiller. These two systems, while differing slightly in their mechanism of temperature control, utilise similar designs in terms of waste removal and water flow, and each has continuously supported CWCs from September 2009 up until the present day. A detailed description of this system is available in Lunden et al. (2014a). In support of ongoing research activities in the Gulf of Mexico (GoM), the recirculating aquaria at Temple were designed primarily to maintain scleractinian CWCs for global ocean change and anthropogenic disturbance studies (e.g. Lunden et al. 2014b; Georgian et al. 2016a, b; Kurman et al. 2017). Now, these aquaria house octocorals being used in further studies of OA, and additional *L. pertusa* colonies for use in oil and dispersant exposure experiments.

Other than all previous examples, Temple University uses artificial seawater (ASW) in its facility. In a recirculating system at an institution far from the sea, this is a necessary and ongoing expenditure; however, ASW usage for water changes can be reduced by efficient filtration systems. The filtration system at Temple University includes a protein skimmer, live rock, Jaubert plenum, and UV steriliser (Fig. 38.12). A secondary consequence of utilising ASW is an additional need to modify the total alkalinity of the seawater, due to ASW's high buffering capacity. This is particularly necessary for OA stud-

ies. To reduce the total alkalinity, the ASW is treated with strong acid (12.1N HCl) followed by a period of air bubbling to facilitate off gassing of excess CO_2. This method has been effective at producing seawater conditions that approximate the natural environment of deep-water corals (Lunden et al. 2013; Georgian et al. 2016b).

The latest efforts using these systems include maintenance of octocoral species from the GoM. From personal observations, octocorals appear to be much more sensitive to laboratory conditions than scleractinians corals, thus necessitating careful management of water quality, feeding, and flow. Thus far, efforts to sustain octocorals have been successful, aided by the use of improved artificial salts, wave makers, power heads, regular water changes, and target feeding with a variety of food sources.
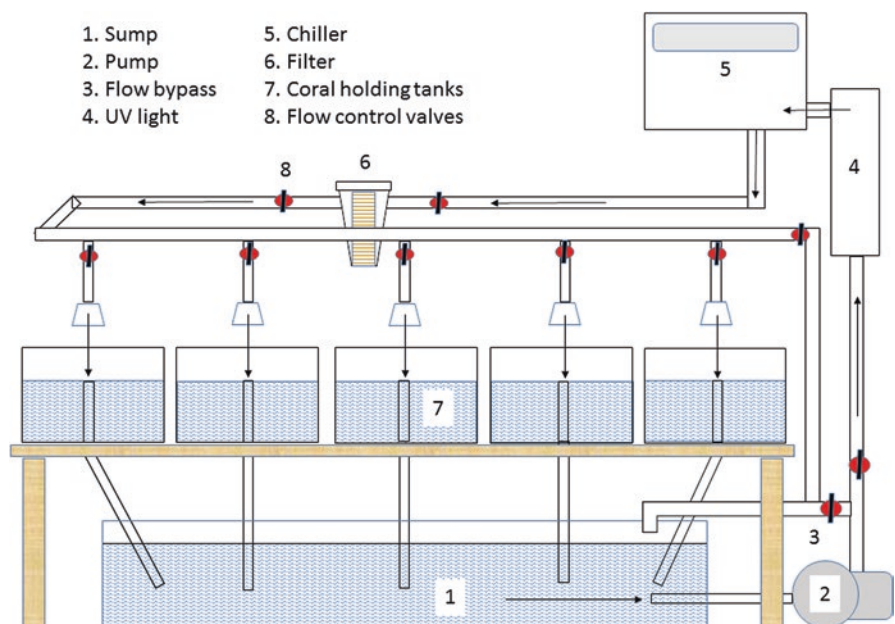
38.3.8 Aquaria Facilities in the Oregon Institute of Marine Biology (Oregon, USA)

The aquaria system in Oregon was initially conceived at Harbor Branch Oceanographic Institution for studies on deep-sea echinoderms, and was subsequently used to maintain *Oculina varicosa*. The system was housed in an insulated building and comprised four independent sets of five tanks, each with a chiller unit that could be programmed for a specific temperature, and a recirculating pump. Since the facility was located at an estuary, water for the system was brought in from offshore and stored in a large (5678 L) recirculating tank until needed. In 2001, this system was relocated to the Oregon Institute of Marine Biology, which is situated on the Pacific coast and therefore has ready access to natural seawater.

Fig. 38.12 Recirculating aquaria at Temple University for CWCs. Top right: holding tank; top left: TECO recirculating chiller; bottom: sump tank with biological and chemical filtration. Reproduced from Fig. 1 of Lunden et al. 2014a *L&O: Methods.* (Photo: J. Lunden, reproduction of the figure authorised by Wiley. © Wiley)

Fig. 38.13 Schematic of a single CWC maintenance tank system operated at Oregon Institute of Marine Biology, showing the lateral view. The different components are labelled and the water flow indicated by arrows. The valves are used to adjust water flow, and for isolating individual tanks for cleaning. (Scheme: © Sandra Brooke)


1. Sump
2. Pump
3. Flow bypass
4. UV light
5. Chiller
6. Filter
7. Coral holding tanks
8. Flow control valves

Each tank series comprises five fibreglass rectangular tanks (1.2 × 0.6 × 0.46 m; 330 L) that are positioned above a larger sump (1.8 × 1.2 × 0.75 m; 1600 L). Water is pumped from the sump through a 0.5 horsepower self-priming pump, through a UV light steriliser and into a chiller. After chilling, the water is pumped into the tanks via a simple filter system, consisting of a household canister style sediment-filter that removes particles >10 μm. Each tank has a feeder line with a pressure disperser on the end to avoid a hard jet of water entering the tank. At the opposite end of the tank, the water leaves through a standpipe and empties into the sump. Valves placed throughout the system are used to control the flow rate, and excess water is bypassed back into the sump (Fig. 38.13). The bottoms of the CWC tanks were covered with a layer of aragonite gravel, and a piece of rigid plastic square mesh (1 × 1 cm × 1 cm deep) was placed on top of the gravel to support the coral fragments and raise them above

the substrate. This arrangement also allowed for moving the corals to a clean tank with minimal handling. The gravel served to maintain appropriate carbonate chemistry, captured any waste organic material (which was then removed during cleaning) and provided a substrate for beneficial bacterial growth.

38.3.9 Aquaria Facilities in the Aquarium Finisterrae (A Coruña, Spain)

The public Aquarium Finisterrae has quarantine facilities as well as research tanks. The receipt of the CWC specimens takes place in the quarantine area, where the newly arrived organisms are acclimated to the aquarium water properties (Fig. 38.14a). Acclimation process is slow and carefully avoiding abrupt changes in the physiochemical

Fig. 38.14 (**a**) *Dendrophyllia cornigera* in a quarantine tank of the Aquarium Finisterrae. The holes on the base are used to provisionally place the colonies to avoid its movement. (**b**) Specimens of *Veretillum cynomorium* in an exhibition tank of the Aquarium Finisterrae. (Photo: © A. Veiga)
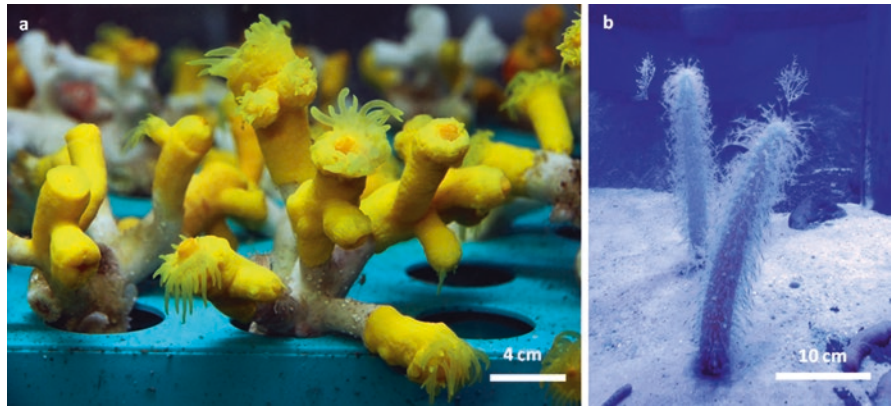
water conditions (especially temperature, pH and dissolved oxygen). The quarantine tanks used in the Aquarium Finisterrae for these animals can hold up to 1400 L, and recirculating pumps are usually placed inside them (whose flow depends both on the volume of the tank and the type of hosted animal). Corals are properly fixed in the aquaria to avoid the displacement of the specimens by the currents inside the tank. In case there is need of cold water input, a heat exchanger or a chiller unit can be used. Nevertheless, the choice of the chilling system will depend on the volume of the tank, of the desired final temperature, as well as the temperature and the input rate of the renewal water. In the acclimation tanks usually used in the Aquarium Finisterrae, specimens of both *L. pertusa* and *M. oculata* were kept for several months in the quarantine zone until they were moved to the exhibition aquaria, where they were kept in similar conditions as the aforementioned. At the Aquarium Finisterrae several species of colonial anthozoan have been maintained such as: some species of the genus *Alcyonium*, gorgonians like *Leptogorgia sarmentosa*, or *Eunicella verrucosa*, sea pens such as *Veretillum cynomorium* (Fig. 38.14b), or the CWCs *D. cornigera*, *L. pertusa*, and *M. oculata*, as well as the anthipatarian *Antipathella subpinnata*. It is important to remark that most of these species also inhabit Mediterranean waters.

The specimens collected for scientific research are placed in polycarbonate aquaria of 40 or 80 L, placed in a small isolated room that has its own water supply and an independent filtering system. This way, the studied material can be subjected to different environmental conditions in a controlled setting. The life support used in this installation has a chilling unit capable of keeping the circulating water at a temperature of 10 ± 0.5 °C, a protein fractionator ("skimmer"), a series of cartridge filters capable of filtering water up to 0.30 µm and an ultraviolet unit placed right before the tank's water input.

38.4 Cold-Water Coral Maintenance

This section has been divided in several subsections to present different experiences on the maintenance of different CWC groups (Box 38.3). Maintenance of scleractinian corals, gorgonians, black corals and sea pens are presented here, some correspond to Mediterranean infrastructures, some to infrastructures located in other European countries or in the USA. A final subsection on coral nubbin preparations for experimental purposes is also included.

38.4.1 Maintenance of Scleractinians

38.4.1.1 A Mediterranean Experience: Long Term Maintenance of four Scleractinian Species

Four different species of hexacorals have been maintained alive for more than a decade in the facilities of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona. These species include *Lophelia pertusa*, *Madrepora oculata*, *Dendrophyllia cornigera* and *Desmophyllum dianthus*. All colonies have been kept in tanks with a capacity between 30 L and 150 L in running natural seawater, which is sequentially filtered (5 µm and 50 µm mesh size), constantly renewed and kept in motion using circulation pumps with a flow rate between 2000 and 4000 L/h. Corals are generally glued onto labelled methacrylate holders using a two-component epoxy resin so they can be kept in an upright position, although the type of support used can vary depending on coral size. Scleractinian colonies are fed according to the size of their polyps. Colonies bearing large polyps, such as *D. cornigera* (20–40 mm in diameter) and *D. dianthus* (15–30 mm in diameter)*,* are generally given commercial frozen krill, *Artemia salina* or *Mysis relicta* shrimps on a daily basis, which are directly supplied to each polyp using a plastic Pasteur pipette after they are

Box 38.3: Best Practices for Scleractinian CWC, Gorgonians and Black Corals Maintenance

There is not a single magic recipe for coral maintenance, as depending on the species and its origin (e.g. geographical region, current regime, depth, temperature) the optimum conditions will vary. However some general aspects have to be taking into account for a successful CWCs maintenance.

– Temperature should be in the range of the area where the corals were collected and kept as constant as possible (Box 38.1)
– Flow through systems are highly recommended as they provide continuous clean water (Box 38.1)
– Water chemistry should be checked daily (ammonium and nitrates should be kept at minimum levels) (Box 38.1)
– Tanks should have at least 50-75 L volume. Tank size depend on the number of nubbins / coral fragments per tank
– Different CWC species can share a tank, but enough distance between nubbins / fragments should be allowed in order to avoid any contact among the polyps
– Scleractinians need high flow rates. A flow rate from 2000 to 4000 L/h is recommended
– Feeding can take place daily, every two days or twice a week.
– Food size and type have to be adapted to the polyp size of the species: Generally frozen krill, *Artemia salina* and *Mysis relicta* are suitable for *Desmophyllum dianthus* and *Dendrophyllia cornigera* whereas copepods (*Cyclops* sp.) and *Artemia salina* nauplii are more adequate for *Madrepora oculata,* gorgonians and certain black coral species (*Antipathes dichotoma*). *Lophelia pertusa* can be fed with *Artemia* naupli, *Mysis* sp.,and also, when available with calanoid copepods. Rotifers are an appropriate food source for species with very small polyp size such us the black corals *Bathypathes* sp. and *Leiopathes glaberrima.*
– Prior to feeding the corals polyps have to be open. If possible manual feeding (using a pipette, syringe etc.) is recommended. If polyps are closed chemical stimulation might be necessary, and can be achieved by preparing a "soup" mixing different food types and releasing it in the aquaria.
– After feeding, the remaining food must be removed.
– As CWC live in the aphotic zone, we recommend maintaining the aquaria in darkness (Box 38.1).

defrosted. It is important that the polyps are fully expanded before starting manual feeding. In general terms, colonies tend to extend their polyps daily if fed with regularity. If this is not the case, a solution composed of a wide range of food particles (1–450 μm) can be diluted in the water tanks to induce polyp expansion. Colonies bearing smaller polyps (5–10 mm in diameter), such as *M. oculata*, are given commercial frozen Cyclops alternated with nauplii of live *A. salina*, which are homogeneously distributed in the water tanks for the polyps to actively capture the prey. No issues have been encountered with mixing different species of scleractinian corals in the same aquaria. However, a minimum distance between colonies is required to avoid any possible contact between polyps when in full expansion. Occasionally, it has been observed that foraminifera may attach to the surface of *L. pertusa* and *M. oculata* nubbins. If foraminifera are not manually removed, sediment and particulate matter tend to accumulate in the surface of the colonies and the coenenchyme, which could lead to the death of the tissue and even to the whole colony.

38.4.1.2   Atlantic and Pacific Experiences: Long Term Maintenance of *Lophelia pertusa* and *Oculina varicosa*

Successful maintenance of scleractinians in aquaria demands not only for proper water chemistry to be met, but also sufficient water movement. The rule, drawn from long experience with *L. pertusa*, seems to be that the turbulence should be just below the limit for the polyp's ability to manage (and thus be able to feed): that is, there should be a noticeable strong flutter of the tentacles due to turbulence, but not so high that the polyp is prevented from positioning it's tentacles to catch food. If the tentacles flop around fully at the mercy of the water movement, turbulence is too strong. The preferred system used for maintaining water movement at the University of Gothenburg (UGOT) has been the '*Mississippi*' chambers; These are experimental chambers of 12 L volume within which a flow-through system can be maintained to provide corals with an appropriate water supply, with paddlewheels integrated into the tank, to mechanically maintain turbulence within the tank, whilst not simultaneously creating bubbles, following an *in situ* design first described in Tengberg et al. (2003). These paddles, reminiscent of those used on historic *Mississippi* riverboats, consist of a long axle and 8 or more flat panels running the length of the axis. By rotating the axis, the paddles rotate in the same direction, with the paddles each displacing water in the same direction in turn; driving the riverboats on the *Mississippi*, or causing elongated displacement waves in the *Mississippi* chambers. These *Mississippi* paddles are placed in the upper portion of the experimental tank, separated by some cm from experimental coral nubbins. These

chambers differ from the initial Tengberg et al. (2003) set-up by facilitating chamber replication with a minimum of mechanical motors. The design of these tanks, and the associated supply systems for dosing tanks continuously with various concentrations of food, pollutants etc. is presented with a schematic drawing in Sects. 38.5.2 and 38.5.4. Although pumps could be used as well, it is important to make sure that no air bubbles are generated since these will stress the corals and trigger mucus production and discharge of cnidae (stinging cells). The pump must also be mounted in a way that minimises vibrations. When using *Mississippi*-type paddles it is important to mount the paddles in a way that minimise surface splashing that also can produce air bubbles and disturbances. The paddles are also a preferred option when corals are kept for breeding, since submerged pumps would destroy the gametes.

Keeping CWCs healthy in aquaria requires a sufficient amount of high quality food, such as calanoid copepods, rich in wax esters and lipids. The brine shrimp *Artemia* sp. is a common food item for corals in aquaria, since they are easily reared and can be served alive. They are, however, probably not sufficiently rich in nutrients (Larsson et al. 2013a) and it is therefore recommended that they be fed with microalgae before feeding them to the corals. There are sources of frozen zooplankton, and at UGOT researchers have used *Calanus* sp., which is a common food source for *L. pertusa* in the North Atlantic (Dodds et al. 2009). Intact frozen copepods have a tendency to float, and therefore the copepods have been homogenised in a blender before feeding. Lipid droplets and carotenoids from the copepods have been observed in histological preparations of mesenterial filaments of recently fed corals (Strömberg and Östman 2016), indicating that they have indeed been feeding on the copepod suspensions.

At UGOT, food is administered twice a week. Too frequent feeding can lead to accumulation of degrading food residues and build-up of biodegrading bacteria and protozoans that can harm the corals, as well as to elevated ammonia and nitrate concentrations, both also harmful for corals. Even though corals potentially can use particulate organic material as a food source, sediment inflow into the aquaria should be minimised to avoid bacterial build-up. Also, the sediments potentially bring other pathogens, such as protozoan parasites or bioeroding organisms. At UGOT researchers use 50- and 5-micron Ametek polypropylene filter cartridges mounted in sequence to filter the incoming water. This system can require frequent filter changes depending on the sediment concentration of the incoming water. A sand filter that is easily back-flushed could probably do the job with less maintenance and at lower cost.

One of the problems when keeping *L. pertusa* in a large aquarium is to perform an efficient feeding of the polyps without altering the water quality due to decaying of accumulated food waste. Mortensen (2001) fed the polyps by adding crushed krill to the water, which rapidly spread with the circulating water through the entire aquarium. In smaller aquaria, it was easier to perform controlled feeding, and to remove food waste after the feeding. Based on this experience, Mortensen considered that the most efficient way of feeding the corals in a small aquarium was to turn off the water supply, and add food above the corals. The food then slowly settles on the polyps. When the polyps have started ingesting the food, the water supply can be turned on again.

In the facilities from the Oregon Institute of Marine Biology, the CWC tanks were maintained at ambient temperature for the target species (16 °C for *Oculina varicosa* and 8 °C for *L. pertusa*). The corals were fed every 3–4 days using live *A. salina* nauplii, which were hatched from cysts in a conical *Artemia* hatchery with seawater and an external light. Prior to feeding, the water flow was reduced to a rate that maintained the appropriate temperature, but did not flush the *Artemia* from the tanks. The corals were allowed to feed for 12–18 h every 4 days, after which time, a Nitex bag (200 μm mesh) was placed over the standpipe outflow to capture unconsumed nauplii, and the water flow was increased. Excess nauplii were removed to maintain low levels of organic material and to prevent formation and accumulation of inorganic wastes, which is particularly important for recirculating systems. This is a simple system with rudimentary water quality control, so in order to maintain healthy animals, every month a 50% water replacement was performed, and a complete system cleaning was done at least every 6 months or more frequently if necessary.

For long term maintenance, flow-through systems are ideal, but not always possible. A series of flow through aquaria have been constructed at the Trondheim Biological Station in Norway (by J. Järnegren, NINA) that are fed by sand-filtered water drawn from 100 m in the Trondheim Fjord. A flow-through system is also available at the UGOT facilities at Tjärnö, Sweden. This system provides a continuous flow of 'ambient' water to the corals, and has proven successful for long-term maintenance and research on early life history studies of *L. pertusa* (see Larsson et al. 2014; Järnegren et al. 2017).

It is also worth mentioning the DyMiCo, active sand bed filter used by R. Osinga and collaborators in the University of Wageningen (The Netherlands). This filter was specifically designed to allow maximal plankton feeding while still having a very high water quality. The system is low maintenance and works quite well for sponges and corals. Dr. Osinga and his team currently have two 3000 L systems for work on boreal deep water sponges. Both systems consist of a water storage tank, a DyMiCo filter and 12 replicate 30 L tanks for experimenting, in which temperature and pH can be controlled individually. This facility is run currently at 6 °C in a climate controlled room.

38.4.2 Maintenance of Octocorals (Gorgonians, Soft Corals and Sea Pens)

In the aquaria facilities of Institut de Ciències del Mar (ICM-CSIC) in Barcelona, several gorgonian species (*Acanthogorgia hirsuta*, *Eunicella singularis, Eunicella cavolini*, *Ellisella paraplexauroides*, *Paramuricea macrospina, P. clavata*, and *Spinimuricea klavereni*) and soft coral (*Alcyonium palmatum*, *Nidalia studeri* and *Paralcyonium spinulosum*) of the Mediterranean continental shelf and slope have been successfully maintained for several years. Acclimation of some of these species to aquarium conditions can be challenging. After placing colonies in different aquaria, polyps of some of these species remained contracted. However, it has been observed that if colonies are placed in large aquaria (over 150 L) equipped with submersible pumps that can generate a flow rate of 4000 L h^{-1}, polyps tend to extend more frequently and acclimation occurs faster. The acclimation period may differ between species, lasting less than a week for some of them (e.g. *E. cavolini* and *P. macrospina*), but can last up to several months in others (e.g. *E. paraplexauroides*).

After acclimation, gorgonians are commonly fragmented into several nubbins for experimental purposes (see Sect. 38.4.4, Fig. 38.16). The coenenchyme at the basal end of the nubbin is removed exposing a portion of the axis (1–2 cm). This denuded portion is covered with specific coral epoxy putty (Coralfix Superfast, Grotech) and the nubbins are attached to an acrylic base. Another system, employed by the DeepSeaLab in Azores, consists in the use of sponge pieces that have previously been sterilised in ethanol and rinsed in distilled water to cover the denude portion. It is extremely important not to cover healthy coenenchyme parts with the coral glue as it may rapidly lead to necrosis of the adjacent tissue (more details in Sect. 38.4.4). In the CWC infrastructures in Barcelona, nubbins of species dwelling on hard substrates are maintained in 30 L aquaria equipped with submersible pumps with flow rates of 2000 L h^{-1}. Also in Barcelona, nubbins and colonies of species dwelling in soft sediments, such as *A. palmatum* and *S. klavereni*, are placed over a layer of fine sands (~15 cm in height) in aquaria of 150 L provided with submersible pumps with flow rates of 4000 L h^{-1}.

Recent experiences with the maintenance of gorgonians (e.g. *Viminella flagellum, Dentomuricea* aff. *meteor, Callogorgia verticillata, Paracalypthrophora josephinae, Acanthogorgia armata*) in the DeepSeaLab in Azores revealed that apart from flow speed, flow direction is very important to keep these species alive. Initially, a single pump with a single outflow tube was mounted in the aquaria and the polyps remained closed for days. However, the installation of a pipe with many holes helped to redistribute the flow into a more uniform laminar way and the polyps opened completely and started feeding normally.

Apart from adequate flow conditions, gorgonians maintained in the Azores appear to be quite voracious requiring abundant and frequent feeding. They are fed every day twice a day (20 mL of food / 30 L aquaria) with a mixture of frozen *A. salina* adults and nauplii, mysids, microplakton, and a food supplement composed of proteins, aminoacids, lipids, vitamins, and oligoelements (Marine Active Supplement, Bentos Nutrition) all partially blended with a food processor. Feeding is supplemented with live rotifers (*Branchionus* sp.) and microalgae (*Chaetoceros* sp. and *Nannochloropsis gaditana*) five times a week.

At ICM-CSIC, most octocoral species are fed with commercially frozen cyclops 3 days a week two times per day. However, it has been observed that this diet is not suitable for *A. hirsuta*, as colonies progressively loose polyps and coloration changes from bright orange to pale grey. After feeding live nauplii of *A.salina* enriched with fatty acids and phytoplankton (*Tetraselmis* sp.) to *A. hirsuta* for several months, its original coloration was restored and polyp loss stopped.

Occasionally, researchers from ICM-CSIC have observed that some nubbins, especially of *E. cavolini* and *E. paraplexauroides*, can be plagued by isopods, which can be removed by submerging the nubbins for 5 s in a brackish solution of 50% seawater and 50% distilled water.

38.4.3 Maintenance of Antipatharians (Black Corals)

The species *Antipathes dichotoma*, *Leiopathes glaberrima* and an unidentified species of the genus *Bathypathes* have been maintained in the aquaria facilities at the Institut de Ciències del Mar (ICM-CSIC) in Barcelona during long periods of time (two years for *A. dichotoma* and *Bathypathes* sp. and six months for *L. glaberrima*). Colonies of these three species are kept in a 30 L aquarium equipped with a submersible pump with flow rates of 2000 L h^{-1} and with 50 μm filtered running natural seawater. In these cases, colonies are not divided into nubbins. Because of the small dimensions of their polyps, finding appropriate food sources for antipatharians has been challenging. After unsuccessfully trying different food regimes (cyclops, live nauplii of *A. salina* and red plankton), it was observed that these organisms can capture live rotifera. In order to supplement their diet, rotifera are enriched with phytoplankton (*Tetraselmis* sp.) and yeast. Antipatharians are fed five times a week two times per day.

The species *Leiopathes* sp. and *Antipathella* sp. are currently maintained at the DeepSeaLab in the Azores. Recent observations of the feeding behavior of these species suggest that they have strong nematocysts and capture food particles easily but take a long time to ingest food because of the slow movement of their tentacles. Based on these observations it is advisable to feed them frequently (at least twice a day) with low amounts of food.

38.4.4 Coral Nubbin Preparation for Experimental Purposes

Coral nubbins, the genetically identical replicates (ramets) generated from a single coral genet, have been proposed as preferred source material for biological and molecular studies. The advantages to work with nubbins are several: sizes can be chosen by the researcher depending on the aquaria and experimental chambers used for different kind of measures to be carried out, further they provide several replicates from a single genetic origin, and finally they also minimise environmental impact by reducing the number of coral colonies collected from the wild. As variability among different clones of one species can be larger than variability between species, it is fundamental to work with distinct batches of at least three different genotypes, so that conclusions can be extrapolated to the species level.

The production of coral nubbins relies on the ability of corals to propagate asexually through fragmentation (Highsmith 1982). Corals often fragment because of physical (storms, strong currents) or biological (predators, bioeroders) factors that break off a portion of the colony. Coral fragments can also be formed by fission, the controlled detachment of coral parts as a form of vegetative reproduction (Lasker 1988). The new fragment is able to survive and form a new independent colony. Cutting techniques or fragmentation follow a similar format. A piece of the parent colony is removed using a cutting instrument, and if the cutting is properly removed, it will heal and form a new colony similar to the parent colony (Borneman and Lowrie 2001).

Nubbins of scleractinian corals can be prepared by cutting coral branches of a parent colony with electrician cutters (or bone pliers) previously cleaned in ethanol. Because of their hard skeletons, coral nubbins can be easily placed in an upright position, i.e. with coral polyps facing upwards by using a plastic mesh or egg crate (Fig. 38.15).

Nubbins of octocorals and black coral can also be prepared by cutting fragments with electrician cutters. However, because of their fragile, flexible structure, these nubbins require a sturdier base to keep the nubbins upright in aquaria. When preparing nubbins, the outer ramets should be selected so that there is only one healing point at the base of the nubbin. Care must be taken to remove the tissue around the axis at the base of the cuttings to avoid it to rot when buried in the epoxy putty.

The researchers from the DeepSeaLab in Azores have developed specially designed bases composed by an "argocrete" support (composed of cement and aragonite sand) and epoxy putty that holds a silicone tube in which the coral fragment is placed (Fig. 38.16). A small piece of artificial sponge inside the silicone tube holds the fragment inside the tubing without damaging it. This base was designed to allow

Fig. 38.15 Plastic basket and mesh (as the ones used in flower shops) to place the nubbins of the scleractinian corals keeping them in an upright position and make the transport easy; in the image the basket contains *Dendrophyllia cornigera* nubbins. (Photo: ©A. Veiga)

the coral to be easily removed and put back in the base. This is particularly useful for experiments that include routine measurements of respiration and buoyant weight, because these measurements can be affected by the biofilm that may form on the holding bases.

38.5 Experimentation in Aquaria: Increasing the Knowledge on the Physiology and Functioning of Cold-Water Corals

This last section of the chapter includes several case studies to present the experience of researchers working in the Mediterranean region and in the Atlantic with different CWC species in aquaria. Using a variety of techniques, different scientific questions have been addressed, such as how CWCs respond to climate change and other environmental variables, and feeding behaviour and reproduction of CWCs. Each subsection comprises a general description of the most common techniques for different types of ecophysiological research on marine invertebrates, application of these techniques to CWCs and a brief description of the main findings.

Comparative experimental studies of corals require that the environment experienced by each coral fragment is similar, except for the studied factor. In the laboratory, for long term studies of physiological behaviour, capture rates, growth rates or spawning of coral species, it is often necessary to resemble the environmental conditions experienced by the sampled coral as accurately as possible. In addition to supplying a simulated or recirculated flux of water to the corals, temperature controlling this water and ideally the laboratory, there are several mechanical options available to simulate flow conditions.

Fig. 38.16 (**a**) Colony of the gorgonian *Dentomuricea* aff. *meteor* being prepared for fragmentation; (**b**) close up of a nubbin and the bases described in the text. (Photos: (**a**) © A. Godinho, (**b**) © R. Sá da Bandeira)

38.5.1 Measuring Growth and Metabolic Responses of Cold-Water Corals Under Different Environmental Conditions

Biomineralisation is a major parameter when addressing effects on the metabolic responses of calcifying species. Calcification rate, together with other important physiological descriptors such as respiration, energy acquisition, fecundity and gene expression, aim to integrate the organism as a whole in order to characterise the main functions affected under different environmental conditions. Skeletal growth is an energy demanding process, which uses a large proportion of the energy acquired by corals. The dynamic energy budget (DEB) model (Kooijman 1986), is a useful tool to estimate the energy needed for calcification and tissue growth according to the environmental conditions in which the corals live. Analysis of skeleton pieces also offers the opportunity to investigate growth on a longer temporal scale, as biogenic carbonates can archive all events that impacted growth during the skeleton formation (Schöne 2008; Montagna and Taviani, this volume). Following this, the use of growth profiles inferred from sclerochronological analysis can help in a precise dating of growth disturbances after the introduction of new environmental parameters.

For CWCs, several methods are used to measure skeletal growth rates in aquaria. These methods differ according to the types of response required (at a global scale or at local spatial scales on the coral fragments) and the duration of experiments (see chapter by Lartaud et al., this volume). Among the main techniques used, the total alkalinity anomaly (TA; Chisholm and Gattuso 1991), inclusion of radioisotopes (e.g., ^{45}Ca or ^{14}C; Tambutté et al. 1995; Hennige et al. 2014) and the buoyant weight (BW, Jokiel et al. 1978; Davies 1989) provide a quantification of the calcification rate in g $CaCO_3$ g^{-1} skeleton day^{-1} or % day^{-1}, the latter method being suitable for experiments at monthly scales. However, it should be taken into account that in case of exponential growth you cannot use "% day^{-1}" as coral size continuously changes (Leal et al. 2016). One important advantage of TA and BW compared to the use of radioisotopes is that the first two are not destructive whereas the latter one is. Estimation of the linear growth extension and sclerochronological tools allows a spatial quantification of growth (in mm year^{-1}). Those methods generally require long term experiments owing to low growth rates of CWCs (see for experiments with Mediterranean CWCs: Orejas et al. 2011; Naumann et al. 2011, 2013, 2014; Lartaud et al. 2013, 2014; Gori et al. 2014b, 2016), although some species have shown visible extension after 2 to 3 months in aquaria or *in situ* (Orejas et al. 2008, 2011; Lartaud et al. 2017a). Finally, quantification of the budding rate, which corresponds to the rate of new polyps formed, can be applied as a measure for growth. These techniques are more relevant for colonial scleractinians and octocorals than for solitary CWCs, as for the former groups the growth is primarily driven by polyp formation (Lartaud et al. 2017b).

Recent CWC research conducted in the facilities of the CSM in Monaco has greatly improved our knowledge on the ecophysiology of Mediterranean CWC. For example, it was shown that these CWC can tolerate much higher growth temperatures than those experienced *in situ* (12 °C), which suggest that they will be able to cope with summer heat waves due to global warming (Naumann et al. 2013). Indeed, several species were able to maintain high calcification rates when cultured for several weeks at 2 °C–3 °C above their normal, *in situ* growth temperatures (Naumann et al. 2014). Studies have also shown the importance of heterotrophic feeding in sustaining high calcification rates of the CWC,

Fig. 38.17 Cylindrical flumes used for feeding experiments. The motor in the top of the chambers is connected to a blade, which keep the water moving at constant flow speed. The speed of the flow can be switched with the controllers (white boxes in the right part of the image). (Photo: © C. Orejas)

also suggesting that CWC are more sensitive to starvation than to temperature increase (Naumann et al. 2011), or the study conducted with *Dendrophyllia cornigera* to evaluate the metabolic response of this coral when it relies on dissolved organic matter as food source (Gori et al. 2014b).

38.5.2 Feeding Experiments with Cold-Water Corals: Closed and Circulating Flumes and 'Mississippi' Chambers

For filter feeding sessile organisms with no algal symbionts, movement within the waters surrounding CWCs is essential for the delivery of food. Flow velocity and reef location influence the food that can be delivered to a reef, given the proximity to a food supply source (such as re-suspended seafloor sediments, fresh phytodetritus or a mixed food supply of phytodetritus and zooplankton). Flow velocity governs the flux and size characteristics of food of different densities or swimming abilities that can be passively transported to a reef, as well as the ease with which particular coral species can collect this carried food from suspension. Passive filter feeders usually show a dome shaped relation between flow speed and capture rate at a given food concentration (e.g. Hunter 1989; Sebens et al. 1997; Allen 1998; Larsson and Jonsson 2006; Wijgerde et al. 2012). With increased flow speed, the flux of food (food per time unit) delivered to the coral increases the feeding efficiency, i.e. the proportion of food particles that can be captured and retained decreases at high flow speeds due to the effect of hydrodynamic forces.

38.5.2.1 Cylindrical Flumes

A modified version of the chambers used by Orejas et al. (2001, 2003) in Antarctica has been utilised by C. Orejas and co-workers in an experiment to find out the ability of

Lophelia pertusa from the Mingulay Reef (Scotland, NE Atlantic) to capture different type of food (copepods, algae, particulate organic carbon) at different current speeds. The chambers consist in 5 L volume cylindrical aquaria equipped with a paddle which is connected to a motor allowing to switch to different current speeds (Fig. 38.17); results revealed a higher feeding rate for this coral species at low current speeds (2–5 cm s^{-1}) varying the efficiency for the different food types (for detailed information on the results of this experiment, please see Orejas et al. 2016.)

38.5.2.2 Circulation Flumes

To the best of our knowledge, the first feeding experiments performed with Mediterranean CWCs were conducted in the aquaria facilities of the CSM in Monaco in 2008. Four CWC species were used in the experiment: *L. pertusa, Madrepora oculata, Desmophyllum dianthus* and *D. cornigera*. *Artemia salina* nauplii and adults were used as prey items at known concentrations, revealing different feeding efficiencies for the four CWC species depending also on the prey size (Tsounis et al. 2010). These first experiments were done using small (850 mL volume) closed recirculation flumes (Fig. 38.18) with a constant unidirectional flow (1 cm s^{-1}) and they took place simultaneously with the experiments carried on with *L. pertusa* by A.I. Larsson, A. Purser and co-workers in the Tjärnö Marine Laboratory in Sweden.

Purser and co-workers (Purser et al. 2010) considered that rather than placing corals in static aquaria or tiny aquaria with tiny mechanical stirring systems, placing corals in circulating flumes could allow a test section (coral mounting area) to be exposed to unidirectional current at a flow velocity set by the researcher (Fig. 38.19). Flow was maintained within these flumes, as already mentioned, by using a rotary paddle, the speed of which is set manually (Berntsson et al. 2004). Care must be taken in the design to ensure that the paddle and drive

Fig. 38.18 Closed recirculation flume (7 L volume) used at the CSM to conduct feeding experiments. (Photo: © S. Reynaud)
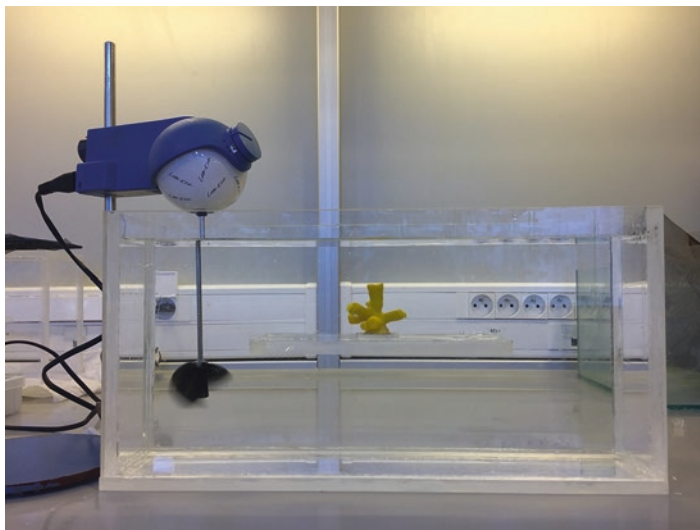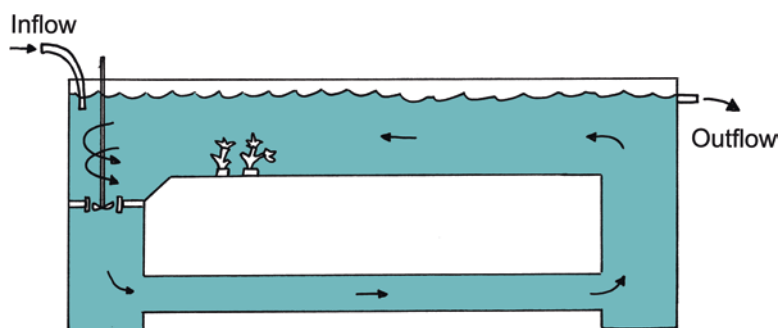

Fig. 38.19 Schematic of a typical 50 L circulation flume. This design offers the possibility for both closed, recirculation functionality and operation as a flow-through system. (Scheme by © A. Purser)

shaft are not constructed of materials that can rust due to the salty seawater used in the experimental runs.

As shown in Fig. 38.19 these flumes can be used as closed systems (as it was the case in Tsounis et al. 2010) or with a continuous flow-through water supply. Commonly, for food uptake studies etc., flumes maintained as flow through systems may have the flow curtailed for the duration of feeding studies, during which the concentrations of food within the 50 L of water are monitored over a known period of time, so that the capture by the corals within the test section can be analysed. Whenever such experiments are based on monitoring the reduction in suspended food over time, it is important to also carry out 'blank' runs, in which comparable 'dead' coral nubbins are used in the place of live corals, to verify that the corals are indeed responsible for suspended food removal over time, rather than a hydrodynamic trapping factor resulting from the coral rugosity. Further, it is also important to consider suspended food removed from suspension by corals may not actually be consumed by the corals (see Sect. 38.5.3). Following an experimental run, the flow-through system can be restarted. Using a circulation flume ensures the flow velocity to be carefully controlled and measured, facilitating comparison of food uptake rates e.g. among flow speeds, within coral species, among different food items at the same flow speeds, and among different species at the same flow velocities. Having well defined and documented flow conditions and food concentrations, is a necessity for comparison of results obtained by independent studies.

These flume designs have been useful in demonstrating that different coral species are able to capture food at varying rates under different flow conditions most optimally (Purser et al. 2010; Tsounis et al. 2010) and that samples of the same coral species, collected from locations with considerably different hydrodynamic and temperature regimes, also collect food at differing rates under differing flow conditions. Larger flumes such as the 1200 L recirculating flume at Tjärnö marine station (described in Jonsson and Johansson 1997) can be used to study flow effects on larger pieces of CWCs or whole coral colonies. This flume has been used to study how *L. pertusa* colonies modify the flow (pattern and velocity) downstream of it, thereby affecting the flux of water and food to neighbouring conspecifics. Further at the CSM, small 7 L volume circulation flumes (Fig. 38.18) have been used in experiments carried out with *D. cornigera* in order to test how the synergy of different factors (e.g. different temperature and flow speed regimes) influence the capture rate ability of this CWC species (Gori et al. 2015).

38.5.2.3 *'Mississippi'* Chambers

Flow conditions surrounding CWCs in the natural situation may be rather chaotic; both as a consequence of the complex relief that may underlie a reef, but also as a result of the complex 3D structure that colonial corals may form with successive generations of growth. The chaotic, turbulent flow can increase feeding rates of corals as the flux and directions of food delivery are varied, and can also help to minimise deposition of material onto coral structure, as may occur under low flow velocity conditions or under unidirectional flow conditions. Turbulent water motion also prevents the development of surface anoxia on fauna and surfaces, an important consideration in long-term or *in situ* experimental studies.

For *in situ* benthic experimental work, experimental chambers deployable as part of lander systems or operated by ROVs, with integrated paddle systems to maintain water movement were developed by the University of Gothenburg for general benthic use (Tengberg et al. 2003). These chambers operate by rotating a *'Mississippi'* type paddlewheel at the top of the chamber by means of a rubber transmission belt, which generates a turbulent flow (see Tengberg et al. 2004). For laboratory work, Jacobs University in Bremen modified the design to allow 5 chambers to be run simultaneously with magnetically linked paddlewheels maintaining equal water movement within 5 separated experimental units (Fig. 38.20). Each chamber contains 12 L of seawater, with either flow-through or static modes possible. Additional experimental functionality is provided by the attachment of peristaltic pumps to each chamber, capable of maintaining the flow of different additional treatments, such as pollutants, food or CO_2 enriched waters (Larsson et al. 2013b). The chambers can also be sealed and used for measurement of respiration rates following treatments. A disadvantage of using this type of chamber is that the flow conditions and hence the flux of food, sediment etc. are not easily measured and defined (will vary with e.g. height of coral, distance from the corners of the chambers), which makes comparisons of results among independent studies difficult.

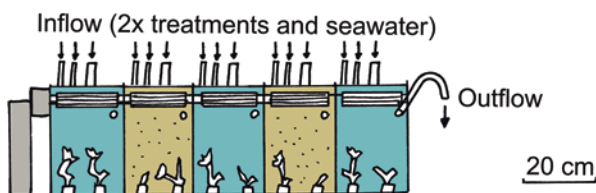At the left of Fig. 38.20 the motor block can be seen adjacent to the paddle in the left-most chamber. This motor is connected through the chamber wall by strong magnets to the first paddlewheel, mounted in the left-most aquaria in a small plastic mount. With both ends of the paddlewheel containing magnets, the remaining paddlewheels are attached in a similar fashion via magnetism through the chamber walls. This allows the 5 paddles to be rotated in unison, with a fixed velocity, throughout the 5 chambers making the experimental unit. Commonly the group have employed 3 × 5 chamber *Mississippi* arrays, to allow 4 × 3 chamber treatments and 1 × 3 control chambers for an experimental run, with the various treatments randomised across the 15 chambers. An alternative to using magnets is to use a standard metal bar running through the 5 chambers, with this bar attached directly to the motor block. With this solution, careful sealing is required to exclude inter-chamber transport of treatments, or the use of a lesser volume of water, i.e. 10 L per chamber, to ensure this axle bar remains above the height of the water in each experimental chamber. With this solution there is a possibility of some bubbles forming during operation, which may have relevance for some treatments or experimental investigations. A further, more complex design can be made by placing the paddlewheels at 90° to those illustrated in Fig. 38.20. In this case, each paddle is connected by magnets to magnets mounted on a drive chain mounted on the outside of the 5 aquaria. In this case, the drive chain turns each exterior magnet ring, which turns the paddlewheel within each chamber (Larsson et al. 2014).

38.5.3 Carbon and Nitrogen Cycling in Cold-Water Corals – The Use of Stable Isotope Tracers

The quantification of carbon (C) and nitrogen (N) resource utilisation and subsequent processing by CWCs remains a challenging task. Traditional gut content analysis is cumbersome, since scleractinian CWCs are not easy to dissect, and their food resources may consist of small-sized particles, dissolved organic and even inorganic compounds (Orejas et al. 2003; Gori et al. 2014b; Mueller et al. 2014; Middelburg et al. 2015). In experimental feeding studies, CWCs were offered a range of food types and their ingestion was subsequently measured as the decrease in food concentration or as the number of food items caught per polyp (Orejas et al. 2003, 2011, 2016; Tsounis et al. 2010; Purser et al. 2010; Gori et al. 2014b, 2015). Nevertheless, some of the trapped food may be lost due to sloppy feeding (Moeller 2005; Pitt et al. 2009) or cannot be assimilated, and thus food uptake rates may not directly translate to assimilation rates. In addition, the coral utilises the assimilated food in its total energy budget (Fig. 38.21), which includes respiration for maintenance and growth, tissue growth and storage, reproduction (Davies 1984; Kooijman 1986), calcification (Cohen and Holcomb 2009; McCulloch et al. 2012) and the release of coral mucus as particulate and dissolved organic matter
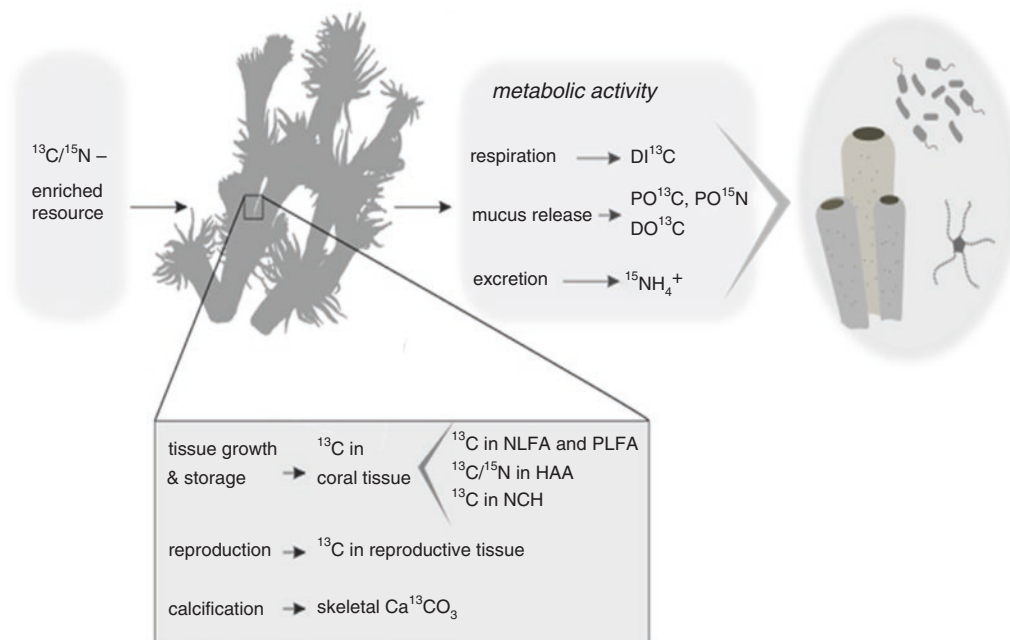

Inflow (2x treatments and seawater)

Outflow

20 cm

Fig. 38.20 Schematic of an array of replicate 10 L *'Mississippi'* chamber aquaria, within which turbulent flow can be maintained whilst providing a flow-through water supply and timed delivery of experimental treatments (Scheme by © A. Purser). (For a photograph of a typical *'Mississippi'* paddle, see Fig. 38.22)

Fig. 38.21 Carbon and nitrogen budget of a cold-water coral feeding on a ¹³C/¹⁵N enriched resource, from assimilation to utilisation for metabolic activity, for tissue and skeletal growth and reproductive tissue formation. The resource ¹³C and ¹⁵N can be 'traced back' in metabolic products such as *DIC* dissolved inorganic carbon, *POC/PON* particulate organic carbon/nitrogen, NH_4^+ ammonium, and into coral (reproductive) tissue plus tissue molecules, *NLFA* neutral lipid-derived fatty acids, *PLFA* phospholipid- derived fatty acid, *HAA* hydrolysable amino acids, *NCH* neutral carbohydrates, and in skeletal calcium carbonate. (Scheme by © S.R. Maier)

(POM, DOM; Crossland 1987; Wild et al. 2008; Naumann et al. 2011; Zetsche et al. 2016). The partitioning of the assimilated C and N amongst these components of the energy budget is difficult to determine.

38.5.3.1   Stable Isotope Tracer Experiments to Unravel Organism Physiology

Pulse-chase stable isotope (SI) studies are used to quantitatively follow the uptake and processing of organic and inorganic resources in freshwater and marine organisms (Middelburg 2014) and represent a promising tool to study C and N cycling in CWCs. The general principle is quite straightforward; an (in)organic C or N resource, with a substantially higher than natural ratio of the heavy isotope (e.g. ¹³C for carbon and ¹⁵N for nitrogen) over the lighter isotope (e.g. ¹²C for carbon and ¹⁴N for nitrogen) is offered to the organism and this 'heavy isotope' pulse is used to trace the fate of the resource in the tissue and metabolic products of a consumer (e.g. Middelburg et al. 2000; Moodley et al. 2000).

SI resources can be obtained in various ways: inorganic resources (e.g. NaH¹³CO₃, or ¹⁵NH₄Cl) can be commercially purchased, while organic resources (e.g. phytoplankton or bacteria) can be produced by culturing them in a ¹³C- or ¹⁵N-enriched medium (e.g. Moodley et al. 2000; Mueller et al. 2014). Enriched dissolved organic material (DOM) can be extracted from an enriched algal culture (de Goeij et al. 2008), while herbivorous zooplankton can be grown by feeding them with enriched phytoplankton (Mueller et al. 2014).

38.5.3.2   Studies on Food Sources, Selectivity and Carbon Budgets

SI tracer studies have shed light on CWC physiology in various ways. Mueller et al. (2014) applied SI experiments to test the ability of *L. pertusa* to exploit a wide range of ¹³C- and ¹⁵N-enriched food substrates, including algal-derived amino acids (DOM), bacteria, phytoplankton and zooplankton. C and N of all the tested resources were assimilated into coral tissue, fatty acids, and amino acids, underlining the opportunistic feeding strategy of CWCs as a potential adaptation to their variable trophic environment. In addition, *de novo* synthesis of individual fatty acids by *L. pertusa* was apparent from the ¹³C enrichment of individual phospholipid-derived fatty acids (PLFAs) in the coral, which were absent in the added food sources. This feature may complicate the interpretation of *in situ* observations on coral nutrition based on lipid composition profiles (Mueller et al. 2014).

van Oevelen et al. (2016) studied selective feeding of *L. pertusa* in a cross-labelling approach, providing corals with ¹³C-enriched phytoplankton *versus* ¹⁵N-enriched bacteria and *vice versa*; they found no selectivity at low food concentrations and a relative preference of phytoplankton over bacteria at higher food concentration.

38.5.3.3   Nitrogen Budgets: From Coral Individuals to the Coral Holobiont

Middelburg et al. (2015) studied the N cycle of the holobiont *L. pertusa* by incubating coral fragments with various inorganic ¹⁵N sources (i.e. ammonium, nitrate and N₂).

Surprisingly, the coral holobiont was able to fix N₂ gas, which is an energetically costly process, advantageous in periods of N shortage in the deep-sea. Moreover, ammonia was nitrified to nitrate by nitrifying bacteria that are likely associated with the coral mucus. The nitrifying community used the energy obtained from ammonium oxidation to fix dissolved inorganic carbon into organic carbon, indicating chemoautotrophic activity. Also, denitrification activity was observed, which was associated with anaerobic parts of the coral gut or with the mucus layer (Middelburg et al. 2015).

38.5.3.4 From the CWC Holobiont to CWC Reefs: Trophic and Non-trophic Interactions

Close trophic interactions between reef species, such as facilitation and recycling of metabolic end products (Fig. 38.21), could represent important reef community adaptations to food or nutrient limitation (Levington 1972; Richter et al. 2001; de Goeij et al. 2013; Rix et al. 2016). The application of SI tracers to follow C and N across several trophic levels revealed, amongst others, the assimilation of coral-released DOM by sponges, and their subsequent shedding of cellular debris as food source for detritivores. This sequence of events has been termed the sponge-loop (de Goeij et al. 2013; Rix et al. 2016). Another study found that the CWC-reef associated polychaete *Eunice norvegica* assimilated two to four times more food-¹³C in the presence of *L. pertusa,* while corals benefitted from the polychaete presence by enhanced calcification (Mueller et al. 2013).

38.5.3.5 Outlook

SI tracer research on CWCs is in its infancy, but can become an important tool, for example to assess changes in energy demand and allocation by CWCs under predicted future ocean scenarios (Cohen and Holcomb 2009). *L. pertusa*'s allocation of food carbon to respiration, mucus production and tissue growth, including the build-up and depletion of carbon stores, has for instance been addressed in a recent SI tracer study

(Maier et al. in press.). Studies on OA and temperature rise could apply the SI tracer methodology to investigate both changes in carbon budgets of CWCs and reef-associated species, and changes in their trophic interactions; assessing the reefs' recycling capacity and resilience.

38.5.4 Experimental Research on Cold-Water Coral Reproduction and Larval Rearing

At the University of Gothenburg (UGOT) *L. pertusa* has been reared for spawning and larval production since 2011 (e.g. Larsson et al. 2014), opportunistically starting in 2009 when spawning occurred during respiration measurements in a metabolic experiment (Larsson et al. 2013a). For spawning purposes, the *Mississippi* chambers developed for previous experiments (see Fig. 38.20, Sect. 38.5.2) were modified: individual chambers were made larger to fit the larger coral fragments needed and the back walls of the chambers were made black so that the whitish eggs could be more easily detected (Fig. 38.22a). The high turbulence from the *Mississippi*-type paddles seemed to stimulate spawning behaviour, and kept the corals in general in a good health.

The parental corals were collected in October–November, a couple of months before the estimated local spawning season in January–February. The early collection was done to allow the corals to acclimatise to the laboratory conditions. The collections were carried out with a landing net that was mounted on an ROV.

38.5.4.1 Gamete Collection and Fertilisation

In *L. pertusa* from the NE Atlantic, oogenesis takes a full year, and somewhat less for spermatogenesis (Brooke and Järnegren 2013). Inducing spawning in corals is difficult (Strathmann 1987), probably since corals do not have oviducts or sperm-ducts, but see Miller (1996) who induced spawning in an antipatharian. Dissecting out polyps to collect gametes is futile due to discharge of cnidocysts that results in

Fig. 38.22 (**a**) Small *Lophelia pertusa* branches in '*Mississippi*' chamber with black wall (**b**) Larger branched colony in darkened '*Mississippi*' chamber. (Photo: © S. Strömberg)
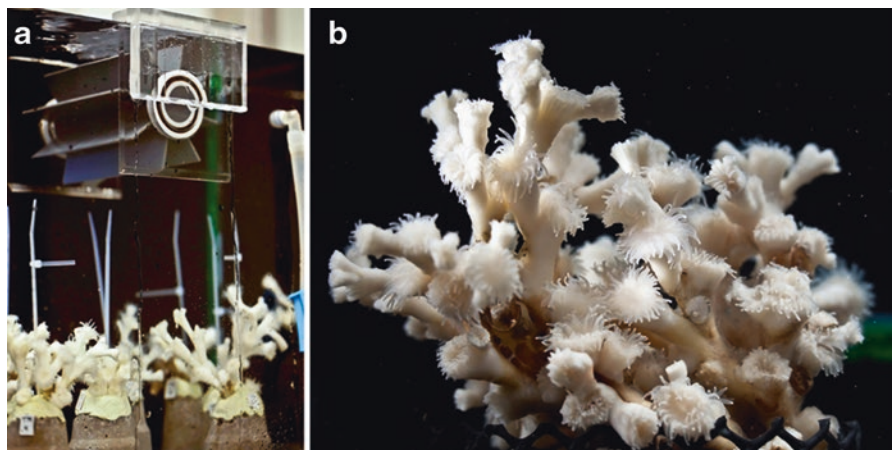
entanglement and destruction. To induce spawning it would probably be necessary to rely on a series of hormones to stimulate final maturation and tissue breakdown, so the best strategy so far is to allow for spontaneous spawning until complete understanding of the reproductive cycle is achieved.

Lophelia pertusa is a broadcast spawner, and spawning in laboratory is protracted over 2 months, possibly with one more intense week during this period. This has yet to be confirmed to also be the case in their natural environment. Spawning by males and females were usually synchronised in chambers where both sexes were combined. Spawning also occurred in chambers where either sex was isolated, although, usually never by both sexes the same day in separate chamber-sets. If isolated eggs and sperm are needed for fertilisation experiments, it is recommended that males and females be placed in adjacent chambers that are interconnected by a membrane or filter that allows chemical signals to pass between them, but not gametes. It is probably conjugated estradiol and testosterone that act as pheromones to synchronise gamete maturation and spawning (Twan et al. 2006); however, this needs further investigation.

Collection of gametes is best done by sucking them up through 5–7 mm tubing, used as a siphon, into large glass bowls for fertilisation and embryo development. The bowl should have a bottom cover of water before transfer. The eggs are approximately 160 μm and barely visible for the naked eye, but they are highly reflective, so with a black background and lateral lighting in an otherwise dark room they are easy to see. Using a sieve to collect the eggs is not possible due to their delicacy; they simply disintegrate in contact with the net, or with surface tension. Eggs and larvae can be handled with glass pipettes, but water motion should always be kept very slow and gentle. Eggs are initially neutrally buoyant and spread out in the entire water mass. After fertilisation they become slightly positively buoyant and start to slowly ascend to just below the surface: usually without getting caught in the surface tension, unless forced into it by too strong turbulence. Larvae will initially actively swim upwards and gather just below the surface as well (Larsson et al. 2014).

If males are less productive and gamete cultures are sperm limited, putting the gamete bowls on a shaking table with gentle motion can increase fertilisation success. The friction of the moving water will increase the water temperature, and development rates will therefore increase if embryos are left on the shaking table as compared with cultures in still water. At UGOT, researchers maintain the cultures, as well as parental corals, at 7–8 °C, since that is the local *in situ* temperatures during the spawning season. They tolerate lower temperatures, however, raising the temperatures to 10 °C or more usually puts the health of cultures at risk due to increased bacterial growth. The *in situ* temperature range for *L. pertusa* and other CWCs is 4–12 °C, however, temperatures above 10 °C have been found to be detrimental in the long run in aquaria.

Besides the work conducted with *L. pertusa,* there are two important papers regarding reproduction and larval ecology of *Oculina varicosa* conducted by Brooke and Young (2003, 2005). Similar data on embryology, larval development, thermal tolerances, and swimming speeds as for *L. pertusa* is thus available also for *O. varicosa*.

38.5.4.2 Larval Rearing

Larval cultures of *L pertusa* have been kept in 2–3 L glass bottles (E-flasks) for maintenance. During the first 3–5 weeks larvae reside in the upper portion of the flasks, swimming underneath the surface, so E-flasks are recommended to be entirely filled up and kept bottom up to give larvae maximum volume. If other types of bottles are used, they can be kept horizontal. Regular changes (e.g. weekly) of a fraction (e.g. 1/3) of the water are sufficient, with occasional larger volumes changed.

After 10 days, larvae are fully developed planulae and good swimmers (see Larsson et al. 2014 and Strömberg 2016 for further details on development). Feeding should start after 20 days—this is when larvae have developed a flexible mouth and are ready for foraging. An oral pore is already visible after 2 weeks, but they are not interested in food at that point. Larvae seem to be opportunistic feeders and prefer a diet similar to adult corals, although, particles of copepods are preferred over live ones. We have homogenised *Calanus* sp., centrifuged the homogenate, and used the fine fraction for feeding. This soup added to cultures has elicited feeding behaviour, that is, larvae swim in a more spiral fashion or stop entirely and move particles towards the mouth by ciliary movements (Strömberg and Larsson 2017). The carotenoids from the copepod fragments are also visible through the body wall of the larvae after feeding, confirming actual intake. Small sized microalgae such as *Isochrysis* sp. and picoplankton have also elicited this behaviour, or trailing of mucus strands that food particles adhere to. Larvae have also been observed to adhere to larger particles of copepods; either feeding directly off the copepod tissues or from the degrading microfauna or picoplankton associated with the tissue.

38.5.4.3 Experiments

The experiments that we have undertaken on *L. pertusa* larvae so far have focused on biological and ecological issues. Specifically, we have tried to establish a timeline for development and ontogenic shifts in behaviour during the pelagic phase of larvae to track what is happening from release to settling. This information is crucial to make adequate projections for larval dispersal, as seen in the work of Fox et al. (2016), where these experimental results were found to contrast with projections based on assumptions of larvae dispersing as passive particles, with fundamentally different outcomes as a result.

To elucidate whether larvae reside in the photic zone during dispersal, we tested if they feed on microalgae, and if they pass through density layers in the water column (Strömberg and Larsson 2017). The latter may be relevant to the Skagerrak area (Northeast Atlantic), where surface waters are affected by the Baltic current, and by outflow from the fjords along the coast, which both give a top layer consisting of less saline water with pronounced haloclines as a result. In most other areas, however, larvae may never encounter lower salinities since offshore oceanic waters usually are not stratified. The larval behaviour in response to salinity gradients was tested in plexiglas aquaria with a bottom slit, allowing for the slow adding of layers of water with different densities. Larvae were then added to the bottom of the aquaria and filmed as they swam upwards. These experiments showed that larvae did not react to salinity differences as high as 5 units between layers, with the top layer salinity as low as 25, instead larvae kept swimming upwards until reaching the surface (Strömberg and Larsson 2017). In addition larvae survived for long even in a salinity of 25 showing that larvae have a broad salinity tolerance range. Tests on feeding preferences also show that larvae might feed on small size microalgae, although this is not fully verified. In summary, we did not find anything that excludes the possibility that larvae spend time in the photic zone during dispersal.

38.5.5 Experiments with Cold-Water Corals under Changing Ocean Conditions

The Changing Ocean Group experimental facility in Edinburgh (Fig. 38.10) has been used for two long-term projects; (1) impacts of ocean acidification (OA) on *L. pertusa* physiology and biomineralisation (Hennige et al. 2015), which ran for 1 year, and (2) impacts of OA on *D. dianthus*, firstly for physiological studies (8 months, Gori et al. 2016) and secondly for biomineralisation research, using pH proxy validation through boron isotopic fractionation (14 months) (Martin et al. 2016). For these experiments, collected corals were fragmented and randomly distributed through all the systems to prevent pseudo-replication. For each of the 5 treatments, there were four replicate systems, each comprising four 5 L tanks connected to a 60 L sump. Each tank was suitable for holding n = 4 live coral fragments and a 'dead' coral skeleton (80 tanks total). Ambient and elevated CO_2 air mixes were bubbled directly into the sump. Experimental conditions for the experiment on *L. pertusa* replicated ambient and predicted future conditions following IPCC emission scenarios. All replicate systems were housed within a temperature-controlled room at ambient reef temperature, and systems at elevated temperatures were controlled through Aqua Medic T-computers and titanium heaters. The bubbled sumps were also equipped

with filtration units and powerheads to ensure adequate filtration and water mixing for each replicate system. *L. pertusa* fragments were fed a mixture of live *Artemia* and crushed krill (Gamma frozen blister packs) and *D. dianthus* were fed frozen mysids every 2 days. For more details on the experimental design see Hennige et al. (2015).

Considering the current literature available, experimental time scales are very important when assessing whether or not corals can acclimatise, as short-term experiments may produce results (for example a detrimental impact of OA upon key processes) that may not appear in longer term studies, as organisms have undergone alterations in key regulatory processes to acclimatise. This makes it very useful to compare both short and long term research, and with regard to *L. pertusa,* most significant changes in respiration and calcification occur in the short term (Hennige et al. 2014, 2015), from 24-h experiments to 4 weeks. Beyond 4 weeks, decreases in calcification and respiration have not been observed in studies to date (see Hennige et al. 2015 and references therein). However, even when acclimatisation has been demonstrated, it may come at a cost to other processes and may therefore not be sustainable in the long-term. Research in the Edinburgh Changing Oceans facility demonstrated that although growth rates can continue as normal under low pH conditions over a period of 12 months, skeletal biomineralisation, molecular-scale bonding and skeletal structure all change. Exposed skeleton cannot acclimatise or adapt to future conditions, and its dissolution is a purely biogeochemical process. The dissolution and weakening of the exposed skeleton observed after long term OA exposure (Hennige et al. 2015) when combined with bio-erosion, may mean that reefs of the future may be smaller than currently, and consequently unable to support the large amounts of biodiversity. The breakdown in the relationship between respiration and calcification in long term experiments may also indicate that 'normal' energetic strategies are circumvented in the long-term, possibly due to other processes using energetic reserves (Hennige et al. 2014, 2015).

Further evidence to support this hypothesis was provided from the study on *D. dianthus*. Whilst these corals may be able to tolerate exposure to acidified seawater, when combined with elevated temperature, respiration and calcification rates decreased. Changes in the ratio of respired oxygen to excreted nitrogen (O:N) were recorded, indicating that the main sources of energy being metabolised shifted from mixed use of protein and carbohydrate / lipid as metabolic substrates under control conditions, to less efficient protein-dominated catabolism under both stressors (Gori et al. 2016). These results support a growing literature consensus that CWCs are amongst the most vulnerable of marine ecosystems to global climatic change (Roberts et al. 2016).

In the same line as the experiments conducted in Edinburgh, J. Movilla and co-workers executed aquaria experiments in the aquaria facilities at the ICM-CSIC, in

Barcelona in order to assess the response of the skeletal structure and the tissue composition to OA of four of the most widely distributed CWC species in the Mediterranean (*L. pertusa*, *Madrepora oculata*, *D. cornigera* and *D. dianthus*; Fig. 38.23). The team developed a system for experimental pH manipulation in aquaria that allowed exposing the organisms to different pH conditions, simulating the present values and those expected by the year 2100. Movilla and coworkers assessed the response of the skeletal structure (calcification rate, microstructure, specific microdensity and porosity) and the tissue composition (organic matter amount and lipids content) in each single species (Movilla et al. 2014a, b; Movilla, this volume).

A pH-manipulative experimental system was implemented based on the experimental design described by Reynaud et al. (2003) (Fig. 38.24). The system was installed inside a temperature-controlled room to ensure constant values during the whole experiment. Seawater was continuously supplied to two 150 L tanks where pH is adjusted to the desired experimental values. Treatment 1 consisted of a pH of 8.10 units (total scale), similar to the current natural pH value observed in the sampling area at similar depths and used as control conditions, while treatment 2 consisted of a pH of 7.81 units, simulating the future Mediterranean decline predicted for the year 2100 following an RCP6 scenario (IPCC2013, AR5). In this experimental set-up instead to bubble a CO_2 air mixes directly into the sump (Hennige et al. 2015), CO_2 (99.9% purity) or CO_2-free air (using a filter filled with soda lime, Sigma Aldrich) were bubbled to either increase or reduce pH, respectively. More details in the experimental design and set-up, as well as in the treatments can be found in Movilla et al. (2014a, b).

For this kind of experiments, it is very important to determine the *in situ* values of the carbonate system in the field as well as to make a good monitoring of these parameters throughout the experimental phase to check the accuracy of the treatments. For that purpose, to compare the control treatment with the natural range that the organisms experience in the field, temperature and salinity profiles were obtained with a Seabird CTD911 from surface to 400 m depth, and water samples for pH and total alkalinity (TA) measurements were taken every 30 m with 24 12-L Niskin

Fig. 38.23 Specimens of *Lophelia pertusa* (above left), *Madrepora oculata* (above right), *Desmophyllum dianthus* (below left) and *Dendrophyllia cornigera* (below right) in the experimental aquaria. (Photos from *L. pertusa* and *M. oculata*: © A. Gori; photo from *D. dianthus* and *D. cornigera*: © E. Obis)
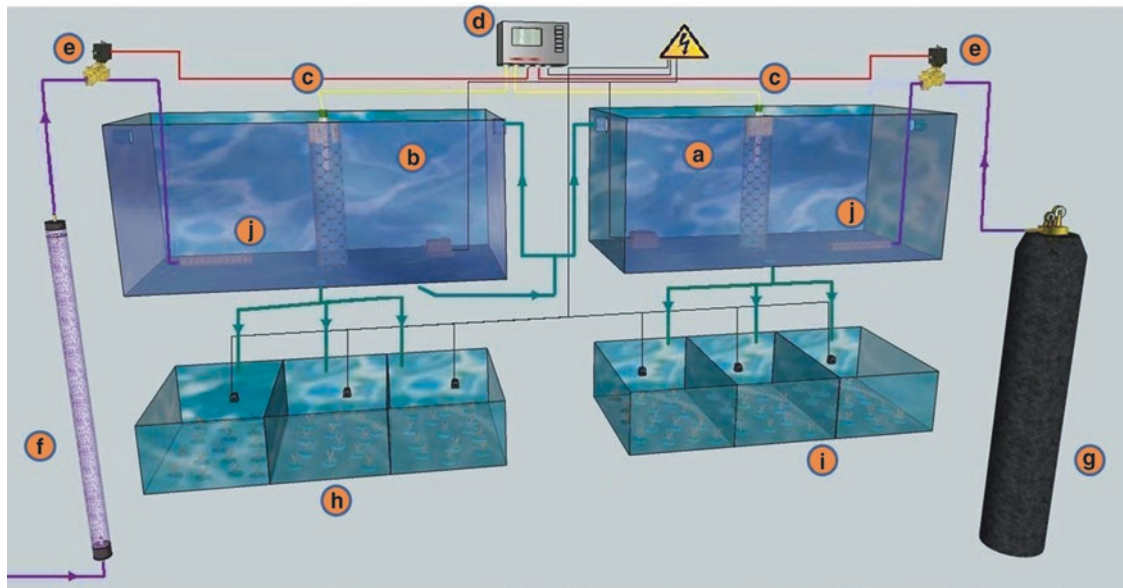
Fig. 38.24 Experimental set-up used to control and modify seawater pH in each aquarium. (**a**) and (**b**) large 150 L tanks for seawater conditioning at pHT 7.81 and 8.10, respectively; (**c**) glass electrodes for pH and PT100 probes for temperature measurements; (**d**) pH controller and data logger; (**e**) solenoid valves; (**f**) soda lime filter; (**g**) 50 kg CO_2 bottle; (**h**) and (**i**) control and low-pH experimental aquaria, respectively (three replicates per treatment); (**j**) Micro bubble diffusers. (Scheme by © J. Movilla)

bottles mounted in a rosette during a research cruise carried out in the sampling area.

In addition, small volumes of water were taken periodically (once a month during the first 3 months and every second month for the rest of the experiment) to analyse TA by potentiometric titration (Pérez and Fraga 1987; Pérez et al. 2000) and pH using spectrophotometry (Clayton and Byrne 1993), which provides better precision than electrodes. These values were used to calculate the rest of parameters of the carbonate system in seawater in both treatments, using the CO_2calc software (Robbins et al. 2010). Results of the experiments have been already published and can be found in Movilla et al. (2014a, b) and Movilla (this volume).

38.5.5.1 Gene Expression Studies with Cold-Water Corals in a Changing Ocean

While the above mentioned studies have mostly focused on studying the effects of OA at organism-level (e.g. growth, calcification, metabolism), only Carreiro-Silva et al. (2014) have looked at the impact of OA at the molecular level of gene expression. For zooxanthellate corals, molecular techniques looking at gene expression have produced useful insights into understanding the physiological pathways involved in the response of corals to OA, by targeting genes involved in the cellular stress response, biomineralisation, and energy metabolism (e.g., Kaniewska et al. 2012; Moya et al. 2012; Vidal-Dupiol et al. 2013). Indeed, the studies of Carreiro-Silva et al. (2014) on the impact of OA on *D. dianthus* have shown that although elevated pCO$_2$ did not cause significant changes in calcification or respiration rates mea-

sured at the level of the organism, gene expression profiles revealed considerable changes in response to OA at the cellular level. The study showed upregulation of genes involved in important cellular processes related to calcification and metabolism as a mechanism to counteract the negative effect of pH on the coral's calcification process. This indicates that CWCs may be able to adjust their physiology in response to environmental changes as a potential mechanism of acclimation or adaptation of CWC to OA.

Consequently, understanding the molecular mechanisms behind the physiological processes involved in a coral's response to elevated pCO$_2$ is critical to assess the ability of CWCs to acclimate or adapt to future OA conditions.

38.5.6 Measuring Effects of Drilling and Oil Spills in Cold-Water Corals

Cold-water corals are often found in association with hydrocarbon drilling areas. Offshore drilling activities discharge large amounts of waste materials into the water column that cause increased sedimentation around oil and gas installations. The discharged drill cutting material is made up of the rock cuttings generated during drilling and attached added drilling fluids (Holdway 2002). For CWC reef fauna, there is great concern is over the potential for drill-cuttings to cause smothering (Roberts et al. 2006), and coral mortality has been observed in the immediate vicinity of drilling discharge points (Gass and Roberts 2006). Another evident risk is oil spills, which, unlike drill cutting discharges, are accidental.

Following the 2010 *Deepwater Horizon* disaster in the Gulf of Mexico, where an amount of oil equal to approximately 4.4 million barrels of oil was released (Camilli et al. 2010), several oil-impacted coral communities were studied *in situ* (White et al. 2012; Fisher et al. 2014). Except for the crude oil, coral communities were also exposed to a chemical dispersant added into the wellhead in order to mitigate the consequences of the oil spill (DeLeo et al. 2016).

There are two main ways of exposing corals to sediments, either by letting the sediment settle onto coral surfaces in water with low or no movement (e.g. Larsson and Purser 2011; Allers et al. 2013) or by (periodically or continuously) exposing the corals to suspended sediment particles in moving water (e.g. Brooke et al. 2009; Larsson et al. 2013b). In the first case with settled sediments, only ordinary aquaria or jars are needed for exposure. Since the surface area of the aquarium floor is known, a slurry of sediment can be added for targeted exposure of sediment mass per unit area. If a certain burial depth is aimed for, pre-tests of resulting sediment depth on the aquarium floor from known sediment loads may be necessary. Flow through of water is turned on after the sediment has settled. Such aquaria experiments have shown that *L. pertusa* actively removes both natural sediment particles and drill cuttings through ciliary movements on the tentacles (Zetsche et al. 2016) and through mucous shedding on tissue covered parts of the skeleton (Allers et al. 2013; Zetsche et al. 2016). Repeated exposure does not affect the cleaning efficiency but sediment can accumulate on tissue-free (bare) skeleton and after repeated exposure cause smothering of adjacent tissue and polyps (Larsson and Purser 2011).

When corals are exposed to suspended sediment particles in moving water, the degree of exposure is depending on the sediment flux, i.e. on the concentration of sediment particles times the flow velocity. For the same sediment concentration, the exposure in the flow direction is hence twice as high when the flow velocity is doubled. The amount of sediment that will end up on vertically and horizontally oriented parts of the coral will depend on the coral morphology, the weight and stickiness of the sediment particles, and the water velocity with more sediment particles settling from above at lower flow velocities. Exposure to suspended sediment particles for a period of time requires appropriate equipment. Brooke et al. (2009) exposed *L. pertusa* fragments to suspended sediments in closed recirculating systems for 2 weeks. Slurry of sediment was introduced at the start of the experiment and the water with suspended sediment was pumped from the conical bottom of the experimental aquarium to the top ensuring water circulation and that sediment particles were kept in suspension. Both Brooke et al. (2009) and Larsson et al. (2013b) regularly monitored the sediment concentration in the experimental aquaria. In Larsson et al. (2013b), *L. pertusa* was exposed to natural benthic sediments and drill cuttings continuously during a period of 3 months (Fig. 38.25). The corals were kept in constant flow through of water in "*Mississippi*" chamber aquaria (Fig. 38.20) and sediments were added to the aquaria by the use of peristaltic pumps from stock solutions with specific concentrations. Sediment particles in the stock solution were kept in suspension by circulation pumps. Constant sediment exposure in the aquaria was reached by balancing the flow through rate of water with the delivery rate of sediment stock solution. The internal circulation of water in the aquaria was governed by the paddlewheels (Fig. 38.20). By maintaining sufficient flow through of water and sediment, the settlement of particles onto corals and other structure in the aquaria was comparatively very small and the particle concentration could be kept constant. Results showed that sediment accumulates also onto vertically oriented surfaces of coral fragments, starting at tissue-free parts, which may result in smothering of polyps. Skeletal growth can correspondingly be slightly adversely affected after months of exposure (Larsson et al. 2013b).

Fig. 38.25 Experimental set-up for long-term exposure of *Lophelia pertusa* to suspended benthic sediments and drill cuttings. Experiment performed at the University of Gothenburg marine station at Tjärnö, Sweden. (Photo: © A. I. Larsson)

38.5.7 The Fragile Chemical Equilibrium in Cold-Water Corals Maintenance: An Example from the Levantine Mediterranean Sea

This section reports a few notes and comments on how an almost catastrophic and irreversible loss of tissue of *Dendrophyllia ramea* colonies kept at the Ocean Aquarium in Cyprus was prevented. The coral colonies were collected off Cyprus at around 150 m depth in a soft bottom habitat. This is the first time that *D. ramea* has been recorded in the Mediterranean Sea at such a remarkable depth and in a sedimentary environment (Orejas et al. 2017).

The aquarium hosting the coral colonies had one ton of seawater with a "sump filtration system". This particular set-up allows for efficient filtration of the aquarium water in a way that the chemical and biological parameters are precisely and fully controlled. Technicians in charge of the coral exhibition performed daily routine checks, such as chemical water analysis and temperature control, to ensure the right aquarium conditions for the corals. Feeding was performed with a syringe, separately to each colony, to minimise food waste and to increase the feeding efficiency of the polyps. Despite the efforts of technicians, 4 months after the corals were on display, an outbreak of filamentous algae was noticed, which infested the whole aquarium within a matter of days. Later on, technicians also observed a sudden and significant regression of live tissue and polyp size and the expansion of filamentous algae on the bare skeleton (Fig. 38.26a, b). An experienced aquarist revealed that the infestation and tissue loss was the result of a sequence of events that occurred due to poor water quality, incorrect lighting (that favors algae proliferation) and insufficient nutrition. The corals were exposed to increased levels of phosphates, and insufficient nutrition, which may have affected growth and calcification. The contracted polyps suggested a weakening of the polyps. Concurrently, increased levels of nutrients (phosphate and nitrate) and excessive illumination exposure of the aquarium promoted the growth of filamentous algae. With the corals weakened and the filamentous algae proliferating in the aquarium, it was a matter of time for the filamentous algae to aggressively colonise the coral skeleton and lead to noticeable coral tissue loss. Once corals reached this health status, it was almost irreversible and the death of the coral colonies was apparently imminent. Technicians, always in close collaboration with the specialist, attempted to limit the infestation using simple, non-invasive techniques. By increasing the frequency of water changes, water quality was rapidly increased with minimal impact on the corals. Although the outbreak was prevented from further infestation, filamentous algae were still present in the aquarium exhibition and the health state of the corals did not change. Improved water quality on its own proved to be a weak treatment and time was running out as more coral tissue was lost during this process.
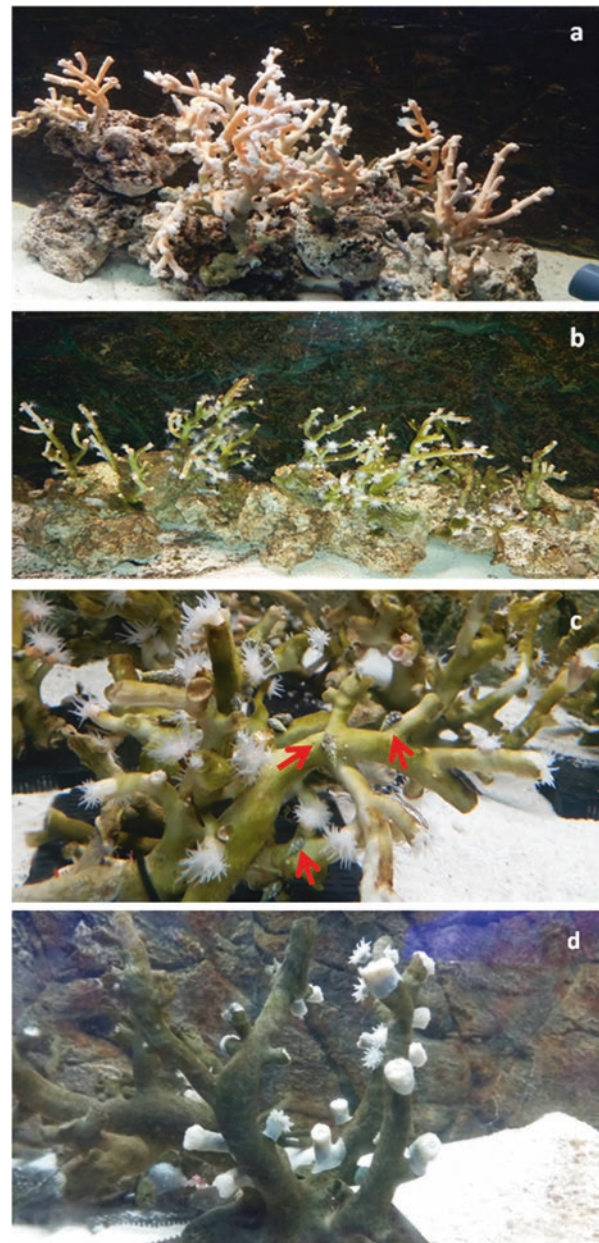

Fig. 38.26 The evolution of the *Dendrophyllia ramea* colonies in the public aquarium of Protaras after an infection event. **a**) healthy *D. ramea* colonies when first transferred in the aquarium facility (June 2015), **b**) colonies already highly infected by the filamentous alga invasion, **c**) *Cerithium* spp. gastropods were introduced in the aquaria to graze the algae, **d**) polyps of *D. ramea* covering again the coral skeleton. (Photos: © V. Andreou)

Hence, it was decided to place the corals in a quarantine tank while the main exhibition tank was disinfected using chemical agents (Fig. 38.26c) to remove the filamentous algae that were still present. This procedure was followed as most of the chemical agents that could be used to eliminate filamentous algae are harmful to most invertebrates and corals in particular. After completion of the disinfection procedure, corals were transferred back to the main exhibition aquarium. Since many variables may have

played a role in the development of the filamentous algae, changes in the aquarium set-up were also performed in order to avoid similar incidents in the future. The original lighting system was replaced with one of a specific wave length that limits photosynthesis, in order to inhibit filamentous algae growth. Additionally, two "wave maker" pumps that simulate alternate underwater currents were installed in the aquarium and the previous UV filtration system was replaced with a larger unit that can circulate water at a higher rate. These were precautionary measures to further enhance coral health.

Despite the efforts of the technicians and the successful removal from the aquarium system of the filamentous algae and cyanobacteria, at this stage the infestation on the coral skeleton persisted and so was the tissue loss. Once the water quality was optimal and monitored regularly, the persistence of filamentous algae on the coral skeletons can be attributed to surrounding lighting. Despite the fact that the lighting unit of the aquarium hosting the corals was replaced, lighting of the adjacent aquariums and the maintenance area behind the aquarium might have provided sufficient lighting for the sustenance of the filamentous algae.

Not being able to treat coral skeleton in the same way as the aquarium (the disinfection procedure is deleterious to living organisms), it was necessary to identify the algae and bacteria (cyanobacteria) that were progressively expanding on coral skeletons. Once identified, a significant number of *Cerithium* spp. gastropods were introduced into the tank with the corals. These benthic species, which were collected from the rocky shoreline in the vicinities of the aquarium, have a specialised diet consisting of different unicellular algae, particularly diatoms, as well as biofilm forming cyanobacteria. A few days after the introduction of *Cerithium* in the tank, the basal sections and branches of the corals exhibited areas free of algae, corresponding to the areas where the gastropods had gathered and grazed-clean the skeleton without affecting the remaining live tissue (Fig. 38.26).

Because of the significant volume loss experienced by the polyps while they were retracted due to the algal infestation, it was attempted to promote recovery by optimising the coral diet. Different feeding types were tested, as well as an increase in the percentage of mysids (small size crustacean) and copepods in the diet. Both groups are common prey for Dendrophyllidae corals. This alternative diet substituted the regular one based on soft tissue without exoskeletons from other decapods and bivalve molluscs. In order to stimulate polyps' capture reaction, fluids and micronised tissue of fresh fish with high fatty acids were added to the water in the tank before the actual feeding with the alternative diet. The polyps' reaction to the fish compounds was positive and immediate: they were ready to capture the mysids that were given individually to each individual polyp with a syringe.

After the successful treatment following the steps mentioned before (regulation of nutrient concentration in the water, lighting, cleaning by the gastropods and a better diet more in agreement with the nature of the Dendrophyllidae species), a significant improvement in the coral colonies was observed. The size of the polyps increased and there was a progressive increase or recovery of lost tissue. At the moment of writing this there is an incipient polyp budding within a few areas of healthy tissue.

Aside from the aspects presented in this chapter regarding the importance of research in aquaria to shed light in the biology, ecology and physiology of CWCs, we would like to stress out the paramount role that exhibitions in public aquaria also have to disseminate the existence and importance of these habitats and communities, and to transmit this message to the society. The dissemination of research is still an unfinished business for many scientist, particularly for the CWC research (see Rossi and Orejas, this volume). Aquaria exhibitions play a fundamental role on this and numerous scientific institutes and public aquaria around the world are starting to contribute to this by planning "open door" days as well as allocating specific areas dedicated to deep-sea fauna.

Some European Research Institutes and Public Aquaria with Aquaria Facilities for Maintenance and/or Experimental Work with CWCs

Cyprus

Ocean Aquarium. P.O. Box 33845, 5318 Paralimni, Cyprus

France

Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique, 66650 Banyuls-sur-mer, France

Germany

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven

GEOMAR Helmholtz Centre for Ocean Research Kiel, Wischhofstr. 1-3, 24148 Kiel, Germany

Italy

Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

Acquario di Genova, Ponte Spinola, 16128 Genova GE

DISVA, Marche Polytechnic University, Via Brecce Bianche, 60131 Ancona, Italy

Monaco

Centre Scientifique de Monaco, Equipe ecophysiologie corallienne, 8 Quai Antoine 1er, MC-98000 Principality of Monaco

Norway

Institute of Marine Research, Austevoll Research Station, 5392 Storebø, Norway

Portugal

IMAR – Institute of Marine Research, University of the Azores, Horta, Portugal & OKEANOS – Center of the University of the Azores Horta, Portugal

Spain

Acuario do Grove, Punta Moreiras, s/n, 36988 O Grove, Pontevedra

Aquarium Finisterrae, Paseo Marítimo Alcalde Francisco Vázquez, 34, 15002 A Coruña, Spain

Institut de Ciències del Mar (CSIC), Pg Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain

Estación de Investigación Jaume Ferrer, La Mola, 07700 Mahón, Menorca, Illes Balears, Spain

Sweden

Department of Marine Sciences, University of Gothenburg, Sweden. Field station on Tjärnö, at the west coast of Sweden and at Kristineberg. Both facilities are run by the Sven Lovén Centre for Marine Infrastructure

The Netherlands

Aquaria facilities in the Wageningen University, Department of Aquaculture and Fisheries, Pots code 338, 6700 AH Wageningen, The Netherlands

United Kingdom

School of GeoSciences, University of Edinburgh, Grant Institute, James Hutton Road, Edinburgh EH9 3FE, UK

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Cross References

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