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Oyster larvae as a potential first feed for small-mouthed ornamental larval fish

Alexander J. Basford*, Benjamin Mos, Tomoki Mishina, Symon A. Dworjanyn

National Marine Science Centre, Southern Cross University, Coffs Harbour, NSW 2450, Australia

ABSTRACT: Aquaculture of ornamental marine fish for the aquarium trade is a possible solution to the negative environmental impacts of wild collection. An impasse to the culture of many marine fish species is high larval mortality due to unsuitable live feeds. Common live feeds can be too large for ornamental species, which often have larvae with small mouths. We tested wild oyster larvae as an alternative live feed for first-feeding blue tang *Paracanthurus hepatus*. *P. hepatus* readily consumed oyster larvae but did not consume rotifers. Survival and growth of *P. hepatus* fed oyster larvae was similar to unfed controls, likely due to the oyster trochophores developing into indigestible veligers. Restricting the diet of *P. hepatus* to only oyster trochophores improved survival and eye development, but survival rates remained low. Oyster trochophores were conditioned in pH 4.8 seawater to compromise their aragonite shells, and *P. hepatus* fed these had higher survival at 5 d post-hatch (dph) compared to those fed untreated trochophores and were the only fish surviving to 10 dph. The high consumption rates of oyster larvae by *P. hepatus* highlight the potential for bivalve larvae to be used as live feeds for first-feeding larval fish. Further research into improving the nutritional value of oyster larvae for larval fish may improve their viability as first feeds.

KEY WORDS: Acidification · Blue tang · *Paracanthurus hepatus* · Fatty acids · Live feeds · Larval rearing

1. INTRODUCTION

The collection of ornamental marine fish for the aquarium trade can cause negative environmental impacts, including decreased biodiversity from overextraction and damage to habitats from destructive fishing practices (Lecchini et al. 2006, Calado et al. 2017). For a growing marine aquarium trade to be sustainable, a greater reliance on cultured fish is essential. A key impasse to the commercialisation of many marine fish species is high mortality during the early larval stage due to a lack of suitable live feeds (Yúfera & Darias 2007, Hamre et al. 2013). Poor first feeds are a common cause of mortality because larval fish have small energy reserves, high metabolic demands, and limited capacity to capture and digest food (Fisher et al. 2007, Yúfera & Darias 2007, Rønnestad et al. 2013). These limitations appear exacerbated in small fish larvae as they are more susceptible to starvation and mortality during the larval stage compared to their larger counterparts (Miller et al. 1988, Pepin 1991, Olivotto et al. 2017).

The early larval stages of many species of marine fish consume only live prey (Dhont et al. 2013). Fish larvae frequently reject artificial diets because movement is required to trigger their feeding response (Langdon 2003, Conceição et al. 2010). Food must also be small enough to be ingested. Fish larvae are only able to capture prey that is 25–60% of their mouth gape (Shirota 1970, Fernández-Díaz et al. 1994, Østergaard et al. 2005). The 2 most common live feeds used in aquaculture, rotifers *Brachionus* spp. and brine shrimp *Artemia* spp., are often too large for fish larvae that have small mouth gapes (<200 µm), such as the early larval stages of acanthurids, chaetodontids, pomacanthids, and some serranids (Nagano et al. 2000a,b, Su et al. 2005, Moorhead & Zeng 2010). A lack of live feeds suited to fish larvae with small mouth

*Corresponding author: alex.basford@scu.edu.au

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gapes is an impediment to closing the lifecycle of many commercially important fish species (Holt 2003).

Attempts to develop live feeds suited to larvae with small mouths have focused on copepod nauplii and, to a lesser extent, ciliates (Nagano et al. 2000a, Støttrup 2000, Olivotto et al. 2005, Ajiboye et al. 2011). Both copepod nauplii and ciliates have the appropriate size (20–90 µm) for consumption by small larval fish (Nagano et al. 2000a, McKinnon et al. 2003). Copepod nauplii are highly nutritious and, when fed to marine fish larvae, improve fish survival and growth compared to common live feeds such as rotifers (Hamre et al. 2008, Olivotto et al. 2008, Karlsen et al. 2015, Zeng et al. 2018). Recent breakthroughs in closing the lifecycles of some ornamental fish species have been facilitated using copepod nauplii as live feeds (DiMaggio et al. 2017, Callan et al. 2018). However, the difficulty and high cost of producing copepods constrains their use in commercial aquaculture (Alajmi & Zeng 2014, Kline & Laidley 2015, Franco et al. 2017). Conversely, ciliates can be easily cultured at high densities but are not nutritious enough to support high larval fish survival (Nagano et al. 2000a,b, Olivotto et al. 2005, de Freitas Côrtes et al. 2013, Leu et al. 2015). The limitations of copepods and ciliates in aquaculture highlight the need to assess the suitability of other small invertebrates as live feeds.

Oyster larvae may be a suitable live feed for small-mouthed fish larvae due to their small size, nutritional profile, and the ease of producing large numbers with minimal cost. Trochophores and early veligers of oyster species such as the cosmopolitan *Crassostrea gigas* and Australasian *Saccostrea glomerata* are similar in size (50–70 µm) to ciliates and copepod nauplii (Table 1). Many oysters are highly fecund, with each female producing millions of eggs (Davis & Chanley 1956, Gallager & Mann 1986, O'Connor et al. 2008). Oysters provision their larvae with high levels of the nutrients that are required by larval fish, including protein and polyunsaturated fatty acids (PUFAs) as both neutral lipids and phospholipids (Chu & Webb 1984, Massapina et al. 1999, Soudant et al. 1999, Caers et al. 2002). Oyster and other bivalve veligers are consumed by fish larvae in the wild and in captivity (Howell 1979, Harding 1999, Cabrera & Hur 2001, Harding et al. 2015, Nack et al. 2015), although survival rates of fish larvae fed oysters vary considerably among species (0–80%) (Howell 1979, Watanabe et al. 1996, Cabrera & Hur 2001). Further research is required to fill knowledge gaps about the potential of oyster larvae as live feeds for many types of fish, such as the larvae of marine ornamental finfish (Oliver et al. 2017).

There is growing interest in developing larval rearing protocols for ornamental fish to offset destructive wild collection (Lecchini et al. 2006, Calado et al. 2017). Many of the fish families that dominate the ornamental trade — Acanthuridae, Pomacanthidae, and Chaetodontidae — have larvae with small mouth gapes and cannot be reliably sourced from aquaculture. Of these fishes, the blue tang *Paracanthurus hepatus* is among the most popular and widely traded, but consumer demand is met completely by wild collection (Militz & Foale 2017, Rhyne et al. 2017). Although there has been some success in closing the lifecycle of *P. hepatus*, high mortality rates during the early larval stage results in few larvae reaching the juvenile stage (Ho et al. 2013, Calado 2017, DiMaggio et al. 2017). Improving the efficacy of live feeds is a key step to overcoming the bottleneck during the early larval stage of *P. hepatus* (DiMaggio et al. 2017).

In this study, we examined the use of oyster larvae as a first feed for small-mouthed larval fish, using *P. hepatus* as a model species. We first tested whether *P. hepatus* consume oyster larvae, and compared growth and survival of *P. hepatus* fed rotifers or oyster larvae. To understand whether different stages of oyster larvae varied in their value as first feeds, we compared the growth and survival of *P. hepatus* fed oyster trochophores that were allowed to develop into the veliger stage with fish larvae fed only trochophores. Finally, in an attempt to improve the digestibility of oyster trochophores, we conditioned them in acidic water to reduce their calcification and compared the survival of *P. hepatus* fed these versus regular oyster trochophores.

2. MATERIALS AND METHODS

2.1. *Paracanthurus hepatus* broodstock

Larval *P. hepatus* were obtained from 9 broodstock housed at the National Marine Science Centre, New South Wales (NSW), Australia (30° 16.05′ S, 153° 08.25′ E). Broodstock were held in an indoor 7000 l recirculating tank at 27.5–29°C, pH 8–8.1, 35 ppt salinity, and given a simulated natural photoperiod. Broodstock were fed daily with a mixed diet of commercial pellets (API® Tropical Pellets, Mars; Hikari Marine-A®, Hikari Seaweed Extreme™, Kyorin Food Industries) and dried seaweed *Porphyra* sp. Broodstock spawned naturally, and floating fertilized eggs were collected overnight in a 50 l egg collector for use in experiments.

Table 1. Sizes of <500 µm planktonic invertebrate larvae that may be considered as live feeds for larval fish. Typical adult fecundity and culture densities are included along with common aquaculture production methods. W: width; L: length; N/A: not applicable. Blank spaces indicate no available data

Common name	Order	Species	Life stage	Production method	Adult fecundity	Culture density	Average size (µm)	Reference
Oyster	Ostreoida	*Saccostrea glomerata*	Trochophore	Spawn adults	$\geq 5 \times 10^6$		55	This study
Oyster	Ostreoida	*Crassostrea gigas*	Trochophore	Spawn adults	$\geq 5 \times 10^6$		70	Helm et al. (2004)
Oyster	Pterioida	*Pinctada margaritifera*	Trochophore	Spawn adults	$\geq 2 \times 10^6$		70	Doroudi & Southgate (2003)
Oyster	Pterioida	*Pinctada fucata*	Trochophore	Spawn adults	$>2 \times 10^6$		75	Rose & Baker (1994)
Scallop	Pectinida	*Nodipecten nodusus*	Trochophore	Spawn adults	$\leq 2 \times 10^6$		90	De la Roche et al. (2002)
Scallop	Pectinida	*Argopecten purpuratus*	Trochophore	Spawn adults			70	Bellonio et al. (1993)
Scallop	Pectinida	*Argopecten irradians*	Trochophore	Spawn adults	$\leq 2 \times 10^6$		80	Sastry (1965)
Scallop	Pectinida	*Amusium balloti*	Trochophore	Spawn adults			85	Rose et al. (1988)
Scallop	Pectinida	*Chlamys asperrimus*	Trochophore	Spawn adults			70	Rose & Dix (1984)
Mussel	Mytilida	*Mytilus galloprovincialis*	Veliger	Spawn adults	$>1 \times 10^6$		105 (W)	Kurihara et al. (2008)
Mussel	Mytilida	*Mytilus edulis*	Veliger	Spawn adults			80 (W)	Gazeau et al. (2010)
Clam	Cardiida	*Tridacna crocea*	Trochophore	Spawn adults			105	Mies et al. (2012)
Clam	Cardiida	*Hippopus hippopus*	Trochophore	Spawn adults	$>1 \times 10^6$		120	Jameson (1976)
Cockle	Veneroida	*Plebidonax deltoides*	Trochophore	Spawn adults	$\leq 4 \times 10^4$		120	O'Connor & O'Connor (2011)
Ciliate	Choreotrichida	*Amphorellopsis acuta*	All	Culture	N/A	<2000 ml^{-1}	95 (L) × 32 (W)	Nagano et al. (2000a)
Ciliate	Choreotrichida	*Tintinnopsis acuminata*	All	Culture	N/A		45 (L) × 20 (W)	Verity (1985)
Ciliate	Choreotrichida	*Strombidinopsis cheshiri*	All	Culture	N/A		70 (L) × 50 (W)	Montagnes et al. (1996)
Ciliate	Choreotrichida	*Tintinnopsis vasculum*	All	Culture	N/A		80 (L) × 45 (W)	Verity (1985)
Ciliate	Choreotrichida	*Lohmanniella spiralis*	All	Culture	N/A		60 (L) × 60 (W)	Jonsson (1986)
Ciliate	Oligotrichida	*Strombidium reticulatum*	All	Culture	N/A		60 (L) × 55 (W)	Jonsson (1986)
Ciliate	Euplotida	*Euplotes* sp.	All	Culture	N/A	<2000 ml^{-1}	34 (L) × 22 (W)	Nagano et al. (2000a)
Ciliate	Prorodontida	*Balanion comatum*	All	Culture	N/A		20 (L) × 15 (W)	Jakobsen & Hansen (1997)
Dinoflagellate	Peridiniales	*Protoperidinium crassipes*	All	Culture	N/A		86 (L) × 80 (W)	Jeong & Latz (1994)
Dinoflagellate	Peridiniales	*Protoperidinium* cf. *divergens*	All	Culture	N/A		73 (L) × 61 (W)	Jeong & Latz (1994)
Tube worm	Canalipalpata	*Spirobranchus giganteus*	Trochophore	Spawn adults	$\leq 1 \times 10^4$		70	Young et al. (2002)
Tube worm	Canalipalpata	*Galeolaria caespitosa*	Trochophore	Spawn adults	$\leq 1 \times 10^4$		100	Marsden & Anderson (1981)
Sponge	Leucosolenida	*Sycon raphanus*	Blastula	Spawn adults			40	Young et al. (2002)
Sponge	Chondrillida	*Halisarca dujardini*	Blastula	Spawn adults			90	Young et al. (2002)
Barnacle	Sessilia	*Hexaminius popeiana*	Nauplii I	Spawn adults			220 (L) × 120 (W)	Anderson et al. (1988)
Barnacle	Sessilia	*Balanus improvisus*	Nauplii I	Spawn adults			202 (L)	Korn (1991)
Barnacle	Sessilia	*Austrobalanus imperator*	Nauplii I	Spawn adults			313 (L) × 173 (W)	Egan & Anderson 1988
Barnacle	Sessilia	*Tetraclitella purpurascens*	Nauplii I	Spawn adults			292 (L) × 172 (W)	Egan & Anderson 1988
Barnacle	Sessilia	*Tesseropora rosea*	Nauplii I	Spawn adults			276 (L) × 147 (W)	Egan & Anderson 1988
Barnacle	Sessilia	*Chthamalus dalli*	Nauplii I	Spawn adults			248 (L) × 138 (W)	Miller et al. (1989)
Barnacle	Sessilia	*Balanus glandula*	Nauplii I	Spawn adults			271 (L) × 163(W)	Barnes & Barnes (1959)
Barnacle	Sessilia	*Megabalanus californicus*	Nauplii I	Spawn adults			394 (L) × 228 (W)	Miller & Roughgarden (1994)
Barnacle	Pedunculata	*Pollicipes polymerus*	Nauplii I	Spawn adults			207 (L) × 114 (W)	Lewis (1975)
Sea urchin	Camarodonta	*Pseudoboletia indiana*	Blastula	Spawn adults			140	Lamare et al. (2018)
Sea urchin	Camarodonta	*Pseodoboletia maculata*	Blastula	Spawn adults			145	Lamare et al. (2018)
Sea cucumber	Aspidochirotida	*Holothuria scabra*	Blastula	Spawn adults	$\geq 4 \times 10^6$		100	Ramofafia et al. (2003)
Sea cucumber	Synallactida	*Stichopus horrens*	Blastula	Spawn adults	$\geq 1 \times 10^6$		200	Hu et al. (2013)
Sea star	Forcipulatida	*Asterias amurensis*	Gastrula	Spawn adults			160	Paik et al. (2005)
Sea snail	Trochida	*Turbo argyrostomus*	Veliger	Spawn adults	$\leq 3 \times 10^4$		190	Kimani (1996)
Sea snail	Trochida	*Trochus niloticus*	Trochophore	Spawn adults	$\leq 1 \times 10^6$		185	Kimani (1996)
Copepod	Calanoida	*Acartia sinjiensis*	Nauplii I	Culture	≤50	≤10 ml^{-1}	75 (L) × 60 (W)	McKinnon et al. (2003)
Copepod	Calanoida	*Parvocalanus crassirostris*	Nauplii I	Culture	≤50	≤10 ml^{-1}	60 (L) × 40 (W)	McKinnon et al. (2003)
Copepod	Calanoida	*Bestiolina similis*	Nauplii I	Culture	≤50	≤10 ml^{-1}	75 (L) × 60 (W)	McKinnon et al. (2003)
Copepod	Harpacticoida	*Tisbe furcata*	Nauplii I	Culture	≤70		62 (L)	Johnson & Olson (1948)
Copepod	Harpacticoida	*Tisbe cucumariae*	Nauplii I	Culture	≤115		72 (L) × 68 (W)	Dahms et al. (1991)
Copepod	Harpacticoida	*Halectinosoma gothiceps*	Nauplii I	Culture			80 (L)	Dahms (1993)
Ostracod	Podocopida	*Dolerocypris fasciata*	Nauplii I	Culture			90 (L) × 60 (W)	Dahms (1993)
Rotifer	Ploima	*Brachionus plicatilis*	All	Culture	1–5	≤1000 ml^{-1}	325 (L) × 240 (W)	Hagiwara et al. (2007)
Rotifer	Ploima	*Brachionus rotundiformis*	All	Culture	1–5	≤1000 ml^{-1}	134 (L) × 102 (W)	Wullur et al. (2009)
Rotifer	Ploima	*Proales similis*	All	Culture	4–8	≤1030 ml^{-1}	109 (L) × 40 (W)	Wullur et al. (2009)
Rotifer	Ploima	*Colurella dicentra*	All	Culture		≤300 ml^{-1}	93 (L) × 49 (W)	Chigbu & Suchar (2006)
Brine shrimp	Anostraca	*Artemia* spp.	Nauplii	Hatch cysts	N/A	≤1 g cyst l^{-1}	400 (L)	Southgate (2012)

2.2. Effects of live feeds on growth and survival of *P. hepatus*

During January 2018, fertilized eggs were transferred from the egg collector to a 12 l aerated incubation tank provided with 0.2 l min^{-1} flow-through seawater (filtered to 1 µm and UV-treated; hereafter FSW), and incubated at 27.5–28°C. At 24 h post-hatch, *P. hepatus* larvae were stocked into 12 l cylindrical black plastic tanks at a density of 100 larvae $tank^{-1}$. Abnormal larvae that could not maintain their position in the water column or were swimming erratically were not used in experiments.

Experimental tanks were kept in heated water baths to maintain a water temperature of 27.5–28°C. Each tank was fitted with a 50 mm ∅ banjo screen with 250 µm mesh that maintained the water volume at 12 l and an airstone providing very fine aeration. Tanks were provided with 0.1 l min^{-1} flow-through FSW until the larvae had developed the ability to feed exogenously at 3 d post-hatch (dph). Water quality parameters during experiments were always within pH 8.0–8.1, >7.5 mg l^{-1} dissolved oxygen, and 35.0–36.0 ppt salinity, measured in all tanks daily using a Hach HQ40d multi-controller fitted with a Hach PHC101 temperature-compensated pH probe, a Hach LDO101 probe, and a Hach CDC101 conductivity probe. The photoperiod was 12 h light:12 h dark, with a light intensity of 2000 lx at the level of the tanks.

Rotifers (*Brachionus* sp., S-strain, Port Stephens Fisheries Institute) were cultured in 2 identical 200 l black fibreglass tanks filled with 28°C, 35 ppt salinity FSW. Each tank of rotifers was fed 50 ml of Nanno 3600 (Instant Algae®, Reed Mariculture) daily and were maintained at a density of 100–150 ml^{-1}. Oxygen saturation was kept above 7.5 mg l^{-1} and 20% of the volume of each tank was exchanged daily. Rotifers were harvested from each tank on alternate days. Rotifers (mean ± SE length = 191.5 ± 2.3 µm, width = 150.2 ± 8.2 µm, n = 50) were washed with FSW in a 45 µm wet sieve before being fed to larval *P. hepatus*.

A total of 60 adult Sydney rock oysters *Saccostrea glomerata* were sourced from a commercial producer at Urunga, NSW, 3 d before each experiment and maintained in tanks containing 25 l of 25–26°C flow-through seawater. Each tank was fed 10 ml of *Pavlova* sp. algal paste (Pavlova 1800, Instant Algae®, Reed Mariculture) daily, during which water flow was stopped for 3 h.

Natural spawning could not be induced, so multiple ripe female and male oysters were strip-spawned as required. Eggs were pooled in a 5 l beaker filled with 0.5 µm FSW. Pooled sperm was added incrementally and fertilization checked microscopically. When >90% of eggs had been fertilized, as evidenced by a fertilization envelope, embryos were added to a cylindro-conical culture tank containing 150 l of gently aerated FSW (25–26°C) for ~10 h to develop to the trochophore stage. Immediately before feeding to *P. hepatus* larvae, oyster trochophores (diameter = 52.6 ± 0.3 µm, n = 50) were washed in a 15 µm wet sieve and counted. When trochophores were required for multiple feeds throughout the day (see Section 2.2.5), trochophores were held in FSW at 4°C for up to 8 h in an aerated 1 l cylindro-conical bottle. This halted the development of the trochophores without causing mortality. Oysters held at 4°C were acclimated at ~27°C for 10 min prior to feeding.

To test the effectiveness of oyster larvae and rotifers as live feeds for newly hatched *P. hepatus* larvae, tanks were randomly assigned to 1 of 4 diet treatments, with 3 replicates $treatment^{-1}$. The 4 diets were oyster trochophores (15 ml^{-1}), rotifers (5 ml^{-1}), rotifers and oyster trochophores (3 and 10 ml^{-1}, respectively), and an unfed control. *P. hepatus* were fed once at the beginning of each light cycle until 5 dph. Prey densities were varied so the biomass among treatments were similar. All tanks were inoculated with live *Nannochloropsis oculata* at a density of 3×10^5 cells ml^{-1} to improve prey contrast and reduce phototaxism (Cobcroft et al. 2012). Tanks were flushed during each dark cycle with 27°C FSW at a rate of 0.1 l min^{-1} to remove all remaining live feeds, algal cells, and waste. No water exchange occurred during the light cycle.

At 3 and 5 dph, 5 larval *P. hepatus* were removed from each replicate 6 h after the beginning of the light cycle, anaesthetized (1 ppm AQUI-S®, New Zealand Ltd), placed on a stage micrometer, and photographed using an Olympus DP26 camera mounted on a stereo microscope. If fewer than 5 larvae remained in a replicate, all fish were sampled. ImageJ 1.51j8 imaging software was used determine whether live feeds had been consumed by measuring the prey incidence and gut area of *P. hepatus* larvae. Prey incidence is expressed as the percentage of larvae that had material in the gut (Pereira-Davison & Callan 2018) (Fig. 1). Notochord length, body depth, and eye diameter were also measured from photographs of the 5 dph *P. hepatus* larvae (Thépot et al. 2016). Survival rates of *P. hepatus* larvae were determined at 5 dph by counting the remaining fish in each replicate and are reported as a percentage of the larvae initially stocked to each replicate tank minus the 5 fish removed from each replicate at 3 dph.

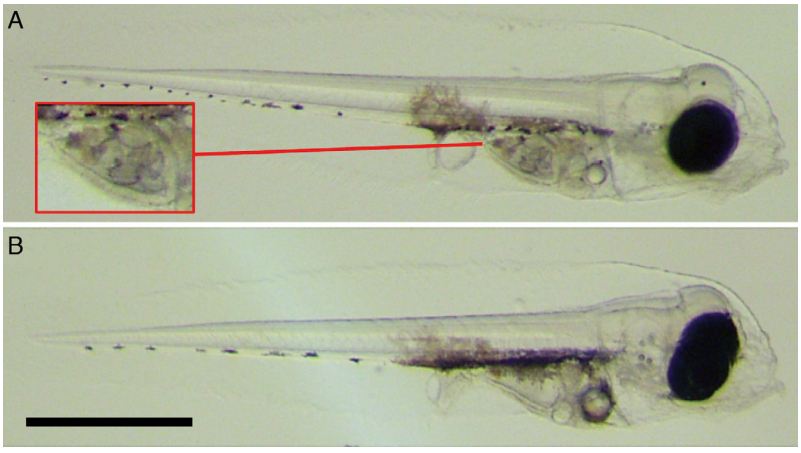

Fig. 1. *Paracanthurus hepatus* larvae 3 d post-hatch exhibiting (A) a gut filled with oyster veligers and (B) an empty gut. Scale bar = 500 µm

2.3. Effects of oyster larval stage on growth and survival of *P. hepatus*

The oyster trochophores developed to the veliger stage after ~6 h in the experimental tanks, and, as veligers possess a calcified shell, this may have reduced their digestibility. To test whether different oyster larval stages varied in their quality as a feed for larval *P. hepatus*, we tested 3 diet treatments using the experimental tanks and setup described in Section 2.2, with 3 replicates treatment^{-1}. The 3 diet treatments were oyster trochophores only, trochophores that were allowed to develop into veligers, and an unfed control. All tanks receiving oyster larvae were fed trochophores at a density of 15 trochophores ml^{-1} at the beginning of the light cycle. Tanks in the trochophore-only treatment were given 2 additional feeds of oyster trochophores 4 and 8 h after the beginning of the day cycle. Replicates receiving additional feeds were flushed for 2 h with 27°C FSW at 0.25 l min^{-1} prior to each feed, which removed >90% of existing trochophores before they could develop into veligers. This flushing did not change the water temperature, pH, or salinity compared to tanks that were not flushed. *N. oculata* was added during the water exchange to maintain the initial cell density. After flushing, the trochophores were restocked at a density of 15 ml^{-1} using trochophores stored at 4°C as previously described. All other treatments received no water exchange during the light cycle. All tanks were flushed each night cycle with 27°C FSW at a rate of 0.1 l min^{-1} to remove all oyster larvae, algal cells, and waste. At 3 and 5 dph, prey incidence and gut area were measured as described in Section 2.2. Similarly, notochord length, body depth, eye diameter, and survival of *P. hepatus* were measured at 5 dph as described in Section 2.2.

2.4. Effect of exposing oyster trochophores to acidic water on survival of *P. hepatus*

During January 2019, fertilized eggs (~12 h post-spawn) were transferred from the egg collector directly into sixteen 12 l cylindrical black plastic tanks filled with 27°C FSW at a density of 600 eggs tank^{-1}. Tanks were set up as per Section 2.2, except there was no aeration. At 24 h post-hatch, tanks were provided with 0.1 l min^{-1} flow-through FSW until *P. hepatus* larvae had developed the ability to feed exogenously at 3 dph. Water quality parameters during experiments were as per Section 2.2. The photoperiod was 16 h light:8 h dark, with a light intensity of 2000 lx at the level of the tanks.

A total of 60 ripe adult *S. glomerata* were sourced from a commercial producer at Urunga the day before the experiment and kept dry at room temperature (~20°C) to prevent spontaneous spawning. Each night prior to feeding, multiple female and male oysters were thermally induced to spawn (O'Connor et al. 2008) in individual 750 ml plastic containers. Gametes were pooled, fertilised, and checked as per Section 2.2 and embryos were added to a 10 l cylindro-conical culture tank containing gently aerated FSW (~25°C) for ~8 h to develop to the trochophore stage. Oyster trochophores were washed in a 15 µm wet sieve, counted, and transferred into four 1 l cylindro-conical aerated bottles filled with FSW; 2 containing 1.1 × 10^6 trochophores, and the other 2 containing 2.2 × 10^6 trochophores. Seawater in one bottle of each trochophore density was reduced to pH 4.8 by addition of 8% HCl solution. This pH was chosen as it rapidly deformed and ruptured trochophore integuments (Fig. 2), indicative of compromised shell calcification, without inducing mortality.

Bottles holding 1.1 × 10^6 trochophores were left at ~25°C for 2 h before the being used for the day's first feed. Bottles containing 2.2 × 10^6 trochophores were held at 4°C for subsequent feeds that day. Oysters held at 4°C were acclimated at ~27°C for 10 min prior to feeding.

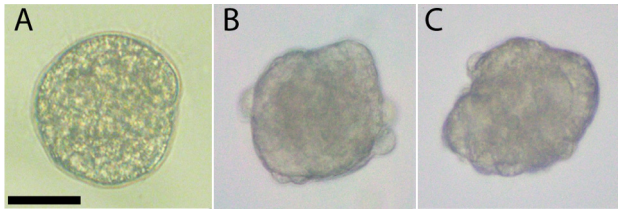

Fig. 2. Typical *Saccostrea glomerata* trochophores exposed to (A) ambient seawater, (B) pH 4.8 seawater for 2 h and (C) pH 4.8 seawater and refrigeration for 12 h. Scale bar = 25 µm

To investigate whether exposing oyster trochophores to acidic water improved their quality as a feed for larval *P. hepatus*, we tested 3 diet treatments with 6 replicates treatment^{-1}. The 3 diet treatments were oyster trochophores exposed to pH 4.8 seawater, trochophores kept in ambient seawater (pH 8.0–8.1), and an unfed control. All tanks were inoculated with live *Chlorella like* at a density of 3×10^5 cells ml^{-1}. All tanks receiving oyster trochophores were fed at a density of 15 trochophores ml^{-1} at the beginning of the light cycle with 2 additional feeds at 5 and 10 h after the initial feed. All tanks were flushed for 2 h with 27°C FSW at 0.25 l min^{-1} prior to each feed, which removed >90% of trochophores before they developed into veligers. *C. like* was added during the water exchange to maintain the initial cell density. After flushing, trochophores were restocked at 15 ml^{-1} using trochophores stored at 4°C as described in Section 2.3. All tanks were flushed each night cycle with 27°C FSW at a rate of 0.2 l min^{-1} to remove all oyster larvae, algal cells, and waste. Survival rates of *P. hepatus* larvae were determined at 5 and 10 dph by counting the remaining fish in each replicate and reported as a percentage of the larvae initially stocked to each replicate tank.

2.5. Statistical analysis

Data for prey incidence, growth, and survival of larval *P. hepatus* were analysed using 1-way ANOVA in SPSS v24.0 with diet as a fixed factor. Normality and homoscedasticity were checked graphically using P–P plots and plots of standardised residuals against predicted values. Data for prey incidence were not normally distributed or homoscedastic, and transforming the data did not have any effect, but given the nature of these data, this was expected. This should be taken into account when interpreting our prey incidence results. Tukey's HSD tests were used when ANOVA indicated there were significant differences among diet treatments ($p < 0.05$).

3. RESULTS

3.1. Effects of live feeds on growth and survival of larval *Paracanthurus hepatus*

At 3 and 5 dph, >75% of *P. hepatus* had prey in their gut when fed either oyster larvae alone or oyster larvae combined with rotifers (1-way ANOVA, 3 dph: $F_{3,8} = 81.22$, $p < 0.001$; 5 dph: $F_{3,8} = 38.42$, $p < 0.001$; Figs. 1 & 3). Up to 6.7% of *P. hepatus* fed rotifers had prey in their guts, while all unfed fish larvae had empty guts (Fig. 3A,B).

At 3 dph, the gut area of *P. hepatus* was larger in fish fed oyster larvae or oyster combined with rotifers compared to fish fed rotifers or unfed controls ($F_{3,8} = 14.45$, $p = 0.001$; Fig. 3C). At 5 dph, the gut area of *P. hepatus* fed oyster larvae was significantly larger than those fed rotifers or unfed controls ($F_{3,8} = 6.96$, $p = 0.013$; Fig. 3D). The gut area of 5 dph *P. hepatus* fed oyster larvae combined with rotifers was not different from all other treatments (Fig. 3D).

At 5 dph, there was no difference in the notochord length ($F_{3,8} = 0.14$, $p = 0.93$), body depth ($F_{3,8} = 0.49$, $p = 0.70$), or eye diameter ($F_{3,8} = 1.95$, $p = 0.20$) of *P. hepatus* among the 4 diet treatments.

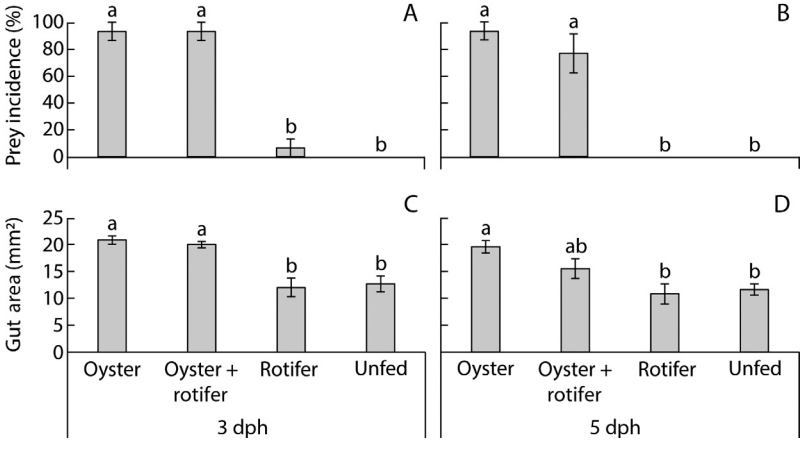

Fig. 3. Effects of diet on (A,B) prey incidence and (C,D) gut area of larval *Paracanthurus hepatus* at (A,C) 3 and (B,D) 5 d post-hatch (dph). Prey incidence: the percentage of larvae with material in the digestive tract. Data are means ± SE, n = 3. Bars with the same letters are not significantly different according to 1-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

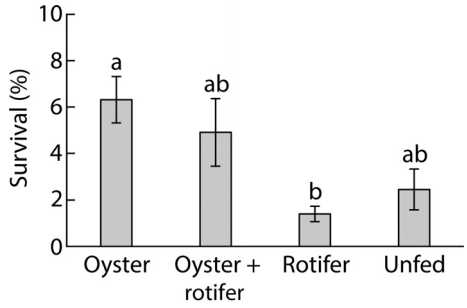

Fig. 4. Effect of diet on survival of *Paracanthurus hepatus* to 5 days post-hatch. Data are means ± SE, n = 3. Bars with the same letters are not significantly different according to 1-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

The survival rate of 5 dph *P. hepatus* fed exclusively oyster larvae ($\bar{x}$ = 6.3%) was significantly greater than those fed only rotifers ($\bar{x}$ = 1.4%) ($F_{3,8}$ = 4.55, p = 0.039; Fig. 4). Survival of *P. hepatus* fed oyster larvae combined with rotifers ($\bar{x}$ = 4.9%) or unfed controls ($\bar{x}$ = 2.5%) was not different from all other treatments (Fig. 4).

3.2. Effects of oyster larval stage on growth and survival of *P. hepatus*

At 3 and 5 dph, >85% of *P. hepatus* larvae had prey in their guts when fed either only oyster trochophores or trochophores that developed into veligers, while all unfed fish had empty guts (3 dph: $F_{2,6}$ = 91.5, p < 0.001; 5 dph: $F_{2,6}$ = 41.52, p < 0.001; Fig. 5A,B).

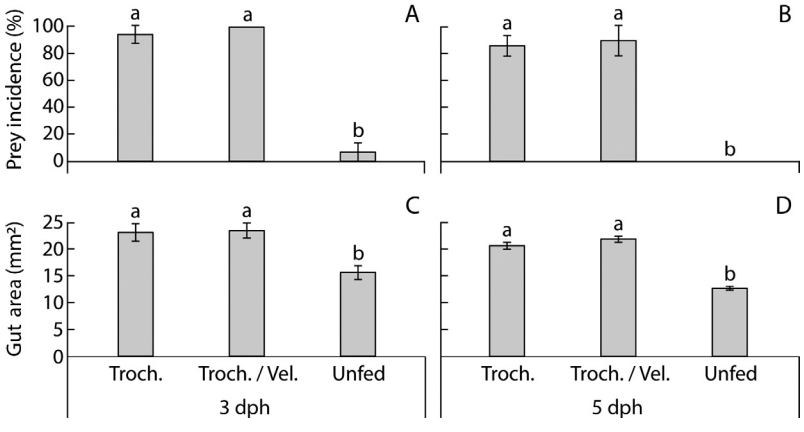

Fig. 5. Effects of diet on (A,B) prey incidence and (C,D) gut area of larval *Paracanthurus hepatus* at (A,C) 3 and (B,D) 5 days post-hatch (dph). Diets: oyster trochophores only (Troch.), oyster trochophores that developed into veligers during the feeding period (Troch./vel.), and unfed. Prey incidence is the percentage of larvae with material in the digestive tract. Data are means ± SE, n = 3. Bars with the same letters are not significantly different according to 1-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

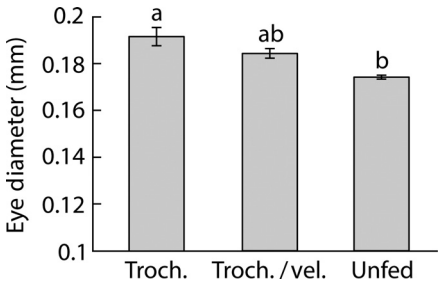

Fig. 6. Effects of diet on the eye diameter of larval *Paracanthurus hepatus* at 5 d post-hatch. See Fig. 5 for further details

At 3 and 5 dph, the gut areas of *P. hepatus* fed only oyster trochophores and trochophores that developed into veligers were not different from each other, but were significantly larger than unfed fish (3 dph: $F_{2,6}$ = 9.34, p = 0.014; 5 dph: $F_{2,6}$ = 85.49, p < 0.001; Fig. 5C,D).

There was no statistical difference in the notochord length ($F_{2,6}$ = 0.88, p = 0.46) and body depth ($F_{2,6}$ = 0.67, p = 0.55) of 5 dph *P. hepatus* among any of the diet treatments. In contrast, the eye diameter of *P. hepatus* fed only oyster trochophores ($\bar{x}$ = 0.191 mm) was significantly wider than unfed *P. hepatus* ($\bar{x}$ = 0.174 mm) ($F_{2,6}$ = 11.40, p = 0.009; Fig. 6). The eye diameter of *P. hepatus* fed oyster trochophores that developed into veligers ($\bar{x}$ = 0.184 mm) did not differ from all other treatments (Fig. 6).

The survival rate of 5 dph *P. hepatus* fed only oyster trochophores ($\bar{x}$ = 9.12%) was significantly higher than unfed fish ($\bar{x}$ = 1.75%) ($F_{2,6}$ = 5.55, p = 0.043; Fig. 7). Survival of *P. hepatus* fed trochophores that developed into veligers ($\bar{x}$ = 5.96%) was not different from any other treatment (Fig. 7).

3.3. Effect of exposing oyster trochophores to acidic water on survival of *P. hepatus*

At 5 dph, *P. hepatus* fed oyster trochophores exposed to pH 4.8 seawater had significantly higher survival ($\bar{x}$ = 41.3%) than those fed regular trochophores ($\bar{x}$ = 13.4%), while unfed fish had significantly lower survival ($\bar{x}$ = 2.6%) than all other treatments ($F_{2,15}$ = 21.39, p < 0.001; Fig. 8A). At 10 dph, the only surviving treatment was *P. hepatus* fed oyster trochophores exposed to pH 4.8 seawater ($\bar{x}$ = 0.33% survival; Fig. 8B).

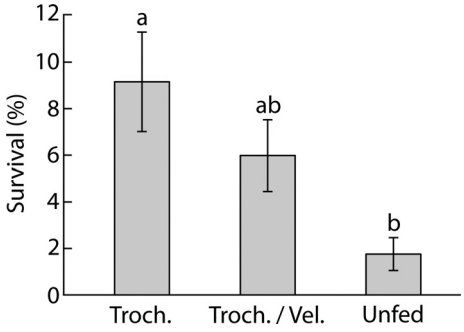

Fig. 7. Effect of diet on survival of *Paracanthurus hepatus* to 5 days post-hatch (dph). See Fig. 5 for further details

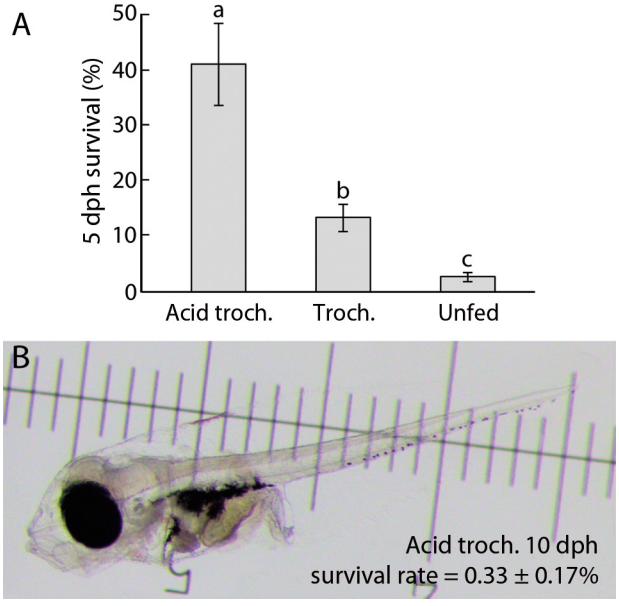

Fig. 8. Effect of diet on survival of *Paracanthurus hepatus* at (A) 5 d post-hatch (dph), and (B) image of 10 dph *P. hepatus* with corresponding survival rate. Diets: oyster trochophores exposed to pH 4.8 seawater (Acid troch.), regular oyster trochophores (Troch.), and unfed. Data are means ± SE, n = 6. Bars with the same letters are not significantly different according to 1-way ANOVA followed by Tukey's HSD test ($p < 0.05$)

4. DISCUSSION

This study tested oyster *Saccostrea glomerata* larvae as a first feed for the commercially important ornamental fish *Paracanthurus hepatus*. We found that *P. hepatus* readily consumed oyster larvae, but almost never consumed rotifers. Survival and growth of *P. hepatus* larvae was poor when fed oyster trochophores that developed into veligers, possibly because veligers have an indigestible shell. The survival of *P. hepatus* was better when fed oyster larvae only at the trochophore stage, and best when oyster trochophores had their calcification compromised in acidic water before being fed to the fish. Despite this promising result, few *P. hepatus* survived beyond 10 dph when fed oyster trochophores, possibly due to nutritional deficiencies. Improving the nutritional quality of oyster trochophores may improve their value as a live feed for larval marine fish.

In this study *P. hepatus* did not eat, and therefore did not survive, when fed rotifers. Rotifers are an established live feed for the larvae of many fish species (Conceição et al. 2010), but are unlikely to be suitable for fish larvae with small mouth gapes that require very small prey (Holt 2003, Moorhead & Zeng 2010, Ho et al. 2013, but see Lee et al. 2018). The rotifers in this study were larger than the mouth gape of larval *P. hepmtus* (110–170 µm; Nagano et al. 2000a, Ho et al. 2013) and were likely too large to be consumed by the early larval stages of *P. hepatus*. This result adds to the growing body of literature demonstrating the unsuitability of conventional live feeds for the small-mouthed larvae of many marine fish (Doi et al. 1997, Su et al. 2005, Moorhead & Zeng 2010, DiMaggio et al. 2017).

Larval *P. hepatus* readily consumed oyster trochophores and veligers. The larvae of a wide range of fish species consume bivalve larvae when offered as a live feed during larviculture (Howell 1979, Harding 1999, Cabrera & Hur 2001, Ma et al. 2013, Harding et al. 2015). Bivalve larvae are rarely found in the guts of wild oceanic fish larvae, probably because bivalves are rare in these environments (Llopiz 2013, Djurhuus et al. 2018). However, in estuaries where bivalves are common, larval fish frequently consume bivalve larvae (Harding 1999, Baker & Mann 2003, Paolucci et al. 2007, Nack et al. 2015). It is likely that *P. hepatus* readily consumed oyster larvae because they were an appropriate size. Larval fish are thought to prefer prey size that is 25–60% of their mouth gape, and the oyster larvae we used were ~30% of the mouth gape of *P. hepatus* (Shirota 1970, Fernández-Díaz et al. 1994, Cunha & Planas 1999, Østergaard et al. 2005). The presence of rotifers did not appear to inhibit oyster larvae consumption, which indicates that larval *P. hepatus*, like other fish species, are able to select appropriately sized prey (Cunha & Planas 1999, Yúfera & Darias 2007).

The swimming behaviour of prey is an important factor in determining the rate at which they are captured and consumed by larval fish. Early studies suggested the conspicuous 'stop-start' movement of copepods was the reason that they are common in wild

larval fish guts (Peterson & Ausubel 1984, Buskey et al. 1993). However, recent research has found that because the early stages of many fish larvae have limited swimming and hunting abilities, they prefer slower-moving prey without predator escape responses (Turingan et al. 2005, Beck & Turingan 2007, Robinson et al. 2019). Oyster larvae display a slow, almost continuous spiralling swimming pattern and were readily eaten by first-feeding *P. hepatus*. A wide spectrum of prey swimming behaviours have triggered successful feeding responses by *P. hepatus* (Lee et al. 2018). This supports the idea that fish larvae do not require that prey have a specific swimming action to trigger a feeding response, and a greater range of organisms than previously thought may be useful as live feeds (Bruno et al. 2018).

Although *P. hepatus* readily consumed oyster larvae, the growth and survival of *P. hepatus* fed oyster trochophores that subsequently developed into veligers was similar to unfed controls. This is similar to Lim (1993) and Cabrera & Hur (2001), who observed poor growth and survival of fish larvae fed exclusively bivalve trochophores and veligers. Conversely, Howell (1979) found high survival (80%) in *Scophthalmus maximus* larvae fed oyster veligers, although larval length and survival was greatest when their diet contained rotifers. The poor survival and growth of *P. hepatus* fed oyster larvae is likely due to the trochophores developing into indigestible shelled veligers (Lee et al. 2006, Ma et al. 2013, Nack et al. 2015). Supporting this, we observed intact veliger shells at the end of the digestive tract of larval *P. hepatus* (Fig. 1A). This problem could be mitigated by flushing veligers out of larval rearing systems and replacing them with newly developed trochophores, as is done with other live feeds when they have lost their nutritional value (Woolley et al. 2012).

We found that *P. hepatus* larvae fed only oyster trochophores, by flushing out oyster larvae before they developed to veligers, had significantly higher survival (9.12%) than unfed controls (1.75%) at 5 dph. This survival is still low relative to other species of similarly aged fish (Olivotto et al. 2006, Fielder & Heasman 2011, Pereira-Davison & Callan 2018), and all fish were dead by 7 dph. We observed that *P. hepatus* were unable to completely digest trochophores (Fig. 9A). While oyster trochophores are less calcified than veligers and do not have a shell, they do have calcified structures and are composed of about 8% aragonite (Lee et al. 2006). We suggest that the rudimentary gut of first-feeding *P. hepatus* is not capable of fully digesting prey with even minimal calcification. Calcification plays an important role in determining the suitability of small invertebrate larvae as live feeds for larval fish.

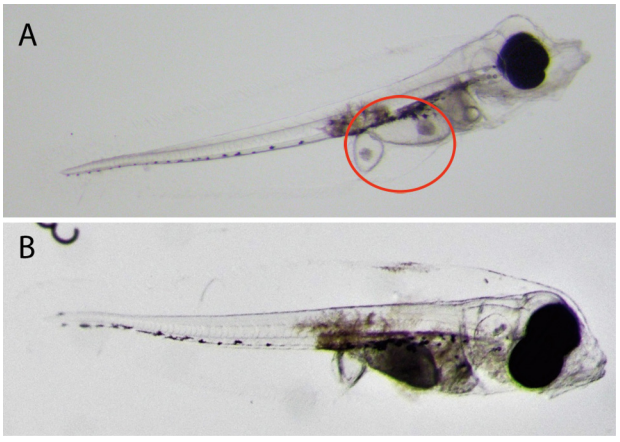

Fig. 9. (A) *Saccostrea glomerata* trochophores (circled) observed swimming in the digestive tract of a 3 day post-hatch (dph) *Paracanthurus hepatus* larvae. (B) 3 dph *P. hepatus* larvae fed oyster trochophores exposed to pH 4.8 seawater. Digestion of trochophores is evidenced by lack of visible intact oysters and homogenous grey matter in the gut

P. hepatus larvae fed only oyster trochophores had significantly greater eye development than larvae that were unfed, but there was no difference in body length or depth of *P. hepatus* among any diet treatments. Eye development is important in larviculture, as it improves the vision and ability of larval fish to capture prey (Yúfera & Darias 2007). Greater opsin protein concentrations and an associated increase in prey capture ability has been shown in 5 dph *Thunnus thynnus* fed diets high in docosahexanoic acid (DHA) (Koven et al. 2018). Bivalve larvae contain high proportions of PUFAs such as DHA, which may explain greater eye development by *P. hepatus* fed trochophores (Caers et al. 2002). However, a substantial increase in eye diameter is usually not seen as early as *P. hepatus* displayed in this study (Roo et al. 1999, Gisbert et al. 2002, Thépot et al. 2016). During the early larval stage, many fish instead prioritise body growth to improve swimming ability (Osse et al. 1997, Yúfera & Darias 2007). Further research is required to understand why *P. hepatus* appears to prioritise growth in eye diameter instead of body size up to 5 dph.

P. hepatus fed oyster trochophores conditioned in pH 4.8 seawater had higher survival compared to fish fed untreated trochophores. The survival rate of 5 dph *P. hepatus* fed trochophores exposed to acidic water exceeds that of yellow tang *Zebrasoma flavescens* fed copepod nauplii to the same age (Pereira-Davison & Callan 2018). While bivalve larvae have been

previously tested as a live feed for fish larvae (Howell 1979, Lim 1993, Cabrera & Hur 2001), this study is the first to use acidic water to compromise oyster trochophore calcification to improve its value as a live feed. Acidic conditions impact the ability of bivalve larvae to calcify and cause abnormalities (Parker et al. 2009, Gazeau et al. 2013). We chose pH 4.8, as it was the lowest pH oyster trochophores could tolerate and caused extreme deformities (Fig. 2). We observed that first-feeding *P. hepatus* were able to digest oyster trochophores exposed to pH 4.8 seawater (Fig. 9B). Exposing oyster trochophores to pH 4.8 seawater facilitated the survival of *P. hepatus* beyond the first-feeding period to 10 dph.

This study highlights the potential for oyster larvae to be used as a live feed for *P. hepatus* and possibly other fish larvae with small mouth gapes. Oyster larvae have the appropriate size and movement to illicit a feeding response by larval fish, challenging the paradigm that larval fish require live feeds like copepods that have a 'stop-start' swimming movement (Bruno et al. 2018). We were able to improve the digestibility of oyster trochophores by conditioning them in acidic seawater. The low survival of *P. hepatus* at 10 dph may be due to the oyster trochophores not providing the fish a nutritionally complete diet, but this requires further research. One method to improve larval fish survival may be to manipulate the diet of oyster broodstock to alter the nutritional profile of their larvae (see the Supplement at www.int-res.com/articles/suppl/q011p657_supp.pdf) (Caers et al. 2002, Uriarte et al. 2004, González-Araya et al. 2012). Future studies should also investigate the biosecurity risks involved with using wild oyster offspring in intensive systems as well as costs associated with maintaining reproductive oyster broodstock year-round for commercial live feed production.

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