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Effect of light intensity and feed density on feeding behaviour, growth and survival of larval sablefish *Anoplopoma fimbria*

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Abstract

We studied the effects of light intensity on larval activity, feeding behaviour, growth and survival of a candidate species for aquaculture – sablefish (*Anoplopoma fimbria*). Of six light intensities ranging from 2 to 750 lux at the water surface, the greatest surviving biomass in small tanks was observed at 12–42 lux. In another experiment in larger tanks, behavioural observations showed that larvae fed poorly under light brighter than 800 lux but fed better at lower light intensities, depending on tank type. In a separate experiment, where live feed densities were varied between 2.5 and 17.5 rotifers per mL of tank water, surviving biomass and dry weight increased with higher feed densities. These experiments help refine methods for rearing larval sablefish by demonstrating effects of light intensity and feed density on larval performance.

Keywords: light intensity, feed density, larvae, sablefish, lux

Introduction

Aquaculture is perhaps most limited by high mortality during larval stages of fish development (Rao 2003). As R-selected organisms, larvae require optimal environmental conditions to thrive and must find and consume enough prey to survive, or risk starvation (Browman 2014). Providing culture environments that maximize larval feed intake will increase production in aquaculture operations (Planas & Cunha 1999).

Marine fish larvae may use chemical cues to detect prey over long distances in nature (DeBose, Lema & Nevitt 2008; Lee, Poretsky, Cook, Reyes-Tomassini, Berejikian & Goetz 2016), but vision likely becomes the most important sensory modality for prey detection and capture at shorter ranges (a few body lengths). In aquaculture, prey are added to rearing tanks at high densities to facilitate consumption. However, prey still must enter the larval visual range before prey can be seen and captured (Blaxter & Staines 1971). Lighting conditions that match the larval visual system should increase visual range, decrease search time, and ultimately increase growth and survival (Aksnes & Giske 1993). Larval visual systems evolve based upon the lighting conditions that are encountered in nature (McFall-Ngai 1990). In aquaculture, lighting conditions must be manipulated to optimize this visual system (Naas, Huse & Iglesias 1996; Mukai & Lim 2014).

Generally, light needs to exceed some minimum intensity threshold before larvae can recognize targets and discern food particles against the prevailing background illumination (Boeuf & Le Bail 1999). Above this threshold, increased light intensity likely follows a normal luminous efficiency function whereby more intense light leads to greater visual function until an optimal brightness is reached, and then, visual performance decreases

as the visual system reaches saturation (Walls 1963). Most species exhibit greater feeding, growth and survival with light intensities greater than 1000 lux, but optimal light intensity can vary according to species and age (Puvanendran & Brown 1998; Boeuf & Le Bail 1999; Planas & Cunha 1999; Ronnestad, Yufera, Ueberschar, Ribeiro, Saele & Boglione 2013; Mukai & Lim 2014; Woolley, Fielder & Qin 2014). Thus, each aquaculture species should be tested to determine optimal lighting conditions.

Another way to maximize feed intake is to increase feed density. Knowing the relationship between feed density and capture rate and their influence upon larval growth and survival will enable aquaculture hatcheries to feed optimally and reduce waste (Puvanendran & Brown 1999; Temple, Cerqueira & Brown 2004). In response to increasing prey densities, encounter rates should initially improve but then plateau or even decrease due to limitations on larval feeding, digestion and prey capture rates (Munk & Kiorboe 1985; Shan & Lin 2014; Ma, Guo, Zhang, Hu & Jiang 2015). As with light intensity, these factors differ among species and should be tested to determine the optimal feed density (Houde & Schekter 1980).

Sablefish (*Anoplopoma fimbria*) is a prime candidate species for marine aquaculture that is rich in omega-3 fatty acids, and has high market value and rapid growth (Sogard & Olla 2001; Gutierrez, Lautenbacher & Hogarth 2007; Friesen, Balfry, Skura, Ikonomou & Higgs 2013; Warpinski, Hermann, Greenberg & Criddle 2016). Sablefish spawn in deep waters (>300 m), and the fertilized eggs slowly rise towards the surface before hatching. Like most marine fish, the larval stage is a major bottleneck for sablefish. The sablefish industry is young, with only a handful of hatcheries that have successfully reared sablefish, and industry standards are still waiting to be developed. To help define industry standards, this study tested the effects of light intensity and feed density on behaviour, growth and survival. Light intensity and feed density treatments were selected to generate a range of values around our current sablefish rearing protocols.

Materials and methods

Details on artificial spawning and rearing can be found in Cook, Massee, Wade, Oden, Jensen, Jasonowicz, Immerman and Goetz (2015). Briefly, for all experiments, wild broodstock were collected and sustained at the Manchester Research Station in 6°C seawater. The broodstock were artificially spawned with one male and one female per cross. Eggs were held under dark conditions from spawning to yolk sac resorption. Headlights with far-red lights were worn while working with the eggs and embryos. After yolk sac resorption at approximately 46 days post fertilization, larvae were transferred to experimental tanks.

Experiment 1. Effects of light on larval behaviour

The larvae used in Experiment 1 were part of a separate experiment that compared rearing success in tanks of different sizes (Cook *et al.* 2015). As such, these experiments were conducted in cylindrical fibreglass tanks of two sizes: two 960-L tanks (104 cm diameter by 152 cm depth, tanks 960-A, 960-B) and three 1920-L tanks (152 cm diameter by 121 cm depth, tanks 1920-A, 1920-B, 1920-C). Cook *et al.* (2015) found that these two tank sizes produced the best growth and feeding, out of four tested tank types. Tank walls were black, but the bottoms were white and flat. The white bottoms aid in being able to see the larvae and clean the tanks (Monk, Puvanendran & Brown 2008). Each tank was stocked at a density of eight larvae per L with larvae drawn from four to six crosses. A stocking density of eight larvae per L is within the range used in commercial sablefish aquaculture and is consistent with previous studies on sablefish larviculture (Cook *et al.* 2015; Lee, Cook, Berejikian & Goetz 2017). Tanks 960-A, 960-B, 1920-A and 1920-B were each stocked over a two-day period, from 21 June 2012 to 22 June 2012. For tank 1920-C, 93% of the larvae were stocked on 18 June 2012 and the remaining 7% were stocked on 22 June 2012. Filtered 12°C seawater entered the tank at the surface and exited via a central standpipe. Each litre of seawater was 'greened' with 0.021 mL of *Nannocloropsis* algal paste (Reed Mariculture, Campbell, CA, USA) and 0.005 mL of green dye (Liquid Color Green Shade, ESCO Food, San Francisco, CA, USA). Including green dye in the greenwater mix has resulted in successful larval rearing in the past with cobia and sablefish (Faulk, Kaiser & Holt 2007; Cook *et al.* 2015). Lighting was provided 24 h per day by one light fixture over the 960-L tanks and two light fixtures over the 1920-L tanks. The fixtures were placed 70 cm above each

tank, and each fixture housed two 1.2-m daylight fluorescent tubes (OctronEco 6500k Sylvania, Danvers, MA, USA). Layers of shade cloth (Easy Gardener, Waco, TX, USA) were inserted into the light fixture between the bulbs and the frosted diffuser cover to control light intensity. Following our usual sablefish rearing methods, two layers of shade cloth were used for the first two days after first feeding (~125 lux at the surface); then, only one layer of shade cloth was used starting the third day after first feeding (~280 lux at the surface).

A waterproof camera (MicroVideo MVC2000 WP-LED, Micro Video Products, Bobcaygeon, ON, Canada) was hung from above the tank with the lens just barely breaking the water surface, halfway between the tank wall and the centre standpipe (Fig. 1). A 24-gauge black-plastic-coated wire was shaped into a 6.5-cm-diameter ring and hung 15 cm below the lens.

After the camera had been in place for 25 min, the number of shade layers was altered in one of the lights (the one closest to the camera in the 1920-L tanks; 960-L tanks only had one light) and 10 min of video was recorded. This routine of altering the number of layers of shade and then recording was continued until we had recorded 10 min for each shade level (0, 1, 2, 3 layers of shade, in randomized order). These shade treatments were selected to generate a range of light intensities around the light intensity that we typically use to rear sablefish. After the last recording, live feed was added to the tank (15 rotifers per mL of tank water), and after 55 min, the recording process was repeated (randomized between 0, 1, 2 and 3 layers). These recordings were taken 10 days (tank 1920-C) to 11 days (all other tanks) after each tank's first stocking date. At this age, the larvae were fully feeding on rotifers and had not yet begun transitioning to *Artemia*. Light intensity was measured at the same location on the water surface as the camera with a portable MW700 lux meter (Milwaukee Instruments, Rocky Mount, NC, USA).

Statistical analyses

To determine the effect of light intensity on swimming activity, we categorized larvae as 'swimming' or 'drifting' during the 'before feed' time periods. We analysed larvae that had any portion of their body above and within the outline of the ring. Larvae were labelled as 'swimming' whenever they contributed to their movement within the tank. This included self-propelled forward movement as well as instances where self-propelled forward movement was interrupted by periods of no motion. 'Drifting' was defined as backwards or sideways movement with no forward motion; that is, movement was primarily by water currents within the tank.

To determine the effect of light intensity on feeding behaviour, we counted the number of larvae that had any portion of their body above and within the outline of the ring every 30 s for 10

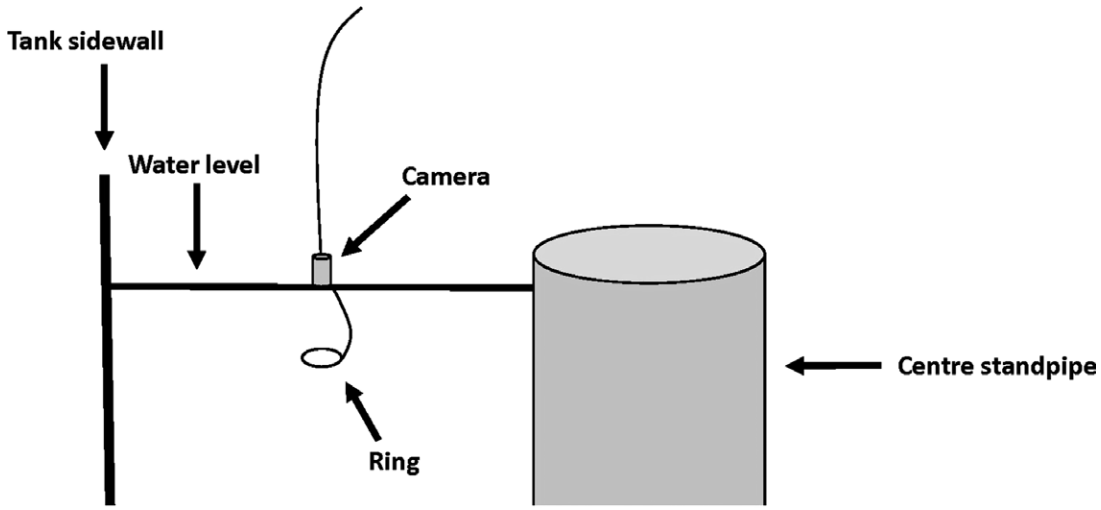

Figure 1 Diagram of the experimental tank in Experiment 1. The camera was positioned halfway between the tank sidewall and the centre standpipe. Tanks were 1920-L and 960-L.

min during the 'feeding' time periods. Like many marine fish larvae, sablefish larvae show a stereotyped feeding behaviour that consists of coiling into a sigmoid body shape then rapidly springing forward to capture prey (feeding strike). For the 'feeding' recordings, we summed the number of feeding strikes across all 30-s periods. We calculated the mean number of strikes per larva by dividing that number by the sum of the number of larvae present at the last second of each 30-s period.

To test whether light intensity and tank size affected activity level or the number of larvae at the surface, mixed models (restricted maximum likelihood, JMP, SAS Institute, Cary, NC, USA) were run with number of larvae seen and per cent larvae swimming as the responses. Light intensity, tank size, and an interaction between light intensity and tank size were fixed effects. As tanks of larvae were randomly rotated through each light intensity treatment, tank was set as a random effect. To test whether light intensity and tank size affected feeding strikes, a mixed model was run with feeding strikes as the response. Fixed effects were light intensity, tank size, and an interaction between light intensity and tank size. Tank was a random effect. For analyses on number of larvae seen, per cent larvae swimming and feeding strikes, only one data point was used per tank, per light intensity. Tanks were rotated among light intensity treatments but were never used more than once within a light intensity (randomized block design).

Experiment 2. Effects of light on growth, survival and surviving biomass

Experiments were conducted in 37-L round tanks (40 cm tall, 37 cm diameter; Fig. 2). Tank walls were black polyethylene. Each tank bottom was originally conical but was converted to a flat bottom by sealing a flat white plastic disc to the tank wall. Filtered 12°C water was supplied from a head tank and manifold to each tank through clear flexible PVC tubing at the tank surface at a rate of 0.25 L per minute, per tank. Water was greened with the same concentrations of algae and dye as in Experiment 1. A series of three nested standpipes maintained a water height of 36 cm while encouraging vertical mixing by forcing water to exit from the bottom of the tank. On top of each tank rested a hollow tube (61 cm tall, 40 cm diameter) made from a sheet of reflective aluminium foil bubble wrap insulation. The bottom end of the tube was open to the tank below. The top end of the tube was covered by a white

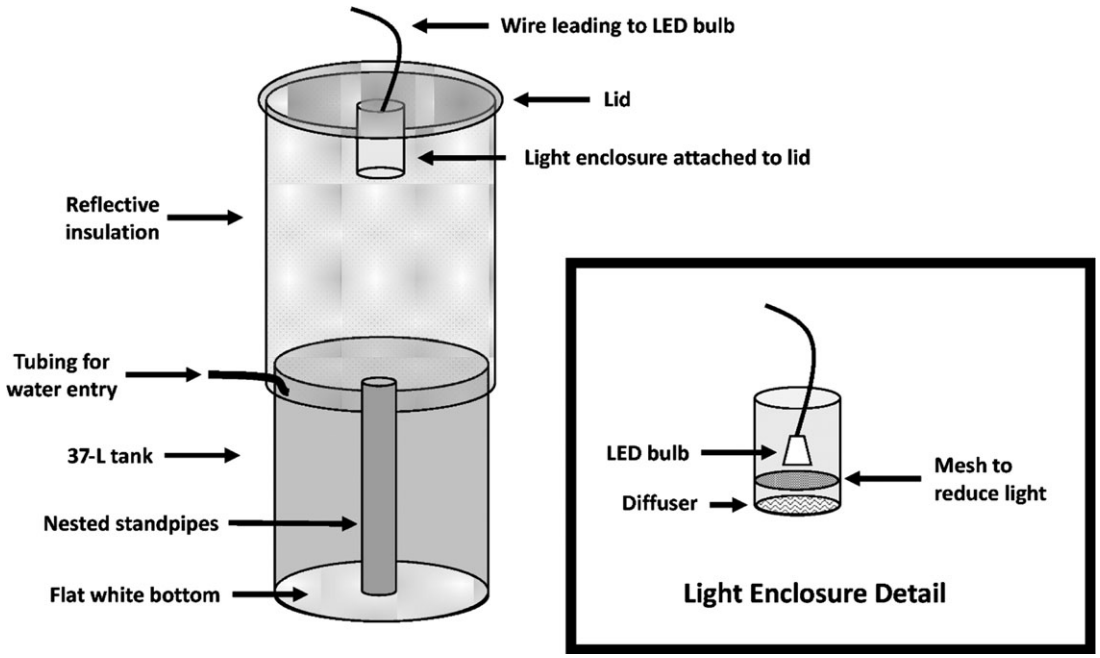

Figure 2 Diagram of the experimental tank in Experiments 2 and 3. Tanks were 37-L tanks with enclosed lights.

plastic lid. The reflective insulation and lid shielded the tank from ambient light. A light enclosure was made of 19-cm-long, 8.3-cm-radius plastic pipe and was attached to the underside of the white plastic disc. The light enclosure pipe housed a LED light bulb (Satco S8813 LED, Brentwood, NY, USA). As the light from the bulb had to pass through the pipe to illuminate the tank, layers of 1.5-mm stainless steel screen mesh were placed in the middle of each pipe as a rudimentary neutral-density filter to control light intensity within the experimental tanks. Light was diffused after passing through the screen mesh within each tank by a piece of UV-transmitting acrylic attached to the bottom of the light enclosure pipe that had been 'fogged' with fine grit sandpaper.

Six light intensities were created by varying the number of layers of mesh in the light enclosure. Light intensity was measured at the water surface, halfway down the tank, and at the bottom of the tank with a MK-9 archival tag (Wildlife Computers, Redmond, WA, USA) and converted to microeinsteins using the equation in Kotwicki, Robertis, von Szalay and Towler (2009). We also converted these readings to lux using an equation derived from readings taken with both the MK-9 and a portable MW700 lux meter (Milwaukee Instruments, Rocky Mount, NC). Light readings are shown in Table 1. We used 1, 6, 10, 14, 18 or 22 layers of mesh, which resulted in light intensities at the water surface of 750, 176, 42, 12, 5 or 2 lux respectively. The experiment was conducted twice. The first trial consisted of 12 tanks ($n = 2$ tanks per light intensity) and utilized the remaining larvae from two crosses that were used for other experimental work at the facility. The second trial consisted of 18 tanks ($n = 3$ tanks per light intensity) and utilized larvae from three crosses that were reared for this project. The tanks from both trials were each stocked with a total of 450 larvae. In the experimental tanks, larvae were fed three times per day at a density of 10 rotifers per mL. After one week for both trials, surviving larvae were anesthetized in MS-222 and counted. Additionally, at the end of the second trial, we also measured the group weight of 30 larvae per tank after drying overnight at 120°C.

Table 1 Conversions between number of layers of light-blocking mesh in the light fixture and light intensity at varying depths in the tank, and between lux and microeinsteins

# Layers mesh	At water surface	Halfway down tank	At bottom
Microeinsteins ($\mu E\ m^{-2}\ s^{-1}$)			
1	20.00	9.05	4.09
6	4.67	1.85	0.84
10	1.09	0.49	0.20
14	0.29	0.12	0.06
18	0.10	0.05	0.02
22	0.03	0.01	0.01
Lux			
1	750	340	154
6	176	70	32
10	42	19	8
14	12	5	3
18	5	3	2
22	2	1	1

Experiment 2 used all light intensity treatments. Experiment 3 utilized only the 14-layer light intensity.

Statistical analyses

A Kruskal–Wallis test with *post hoc* Tukey HSD was used to test for effects of treatment (six light intensities) and trial (trial 1 or trial 2) on survival. During trial 1, one of the 6-mesh tanks went from having apparently normal survival to zero survival over the course of 24 h. This sudden mortality was likely due to a bacterial infestation (indicated by bacterial growth that resembled spider webs in the tank), and therefore, the tank was excluded from analyses. None of the other tanks had this kind of bacterial growth. For trial 2, Kruskal–Wallis tests with *post hoc* Tukey HSD were used to test for differences in group dry weight of 30 larvae and total surviving biomass among treatments, where

$$\text{Total surviving biomass} = \#\ \text{survivors}\left(\frac{\text{dry weight of 30 larvae}}{30}\right).$$

Experiment 3. Feed density

Tanks, light setup, greening and water flow were identical to those used in Experiment 2. Fourteen layers of mesh were used in the light enclosures to generate 12 lux of light at the water surface.

Eighteen tanks were each stocked with 350 larvae from two crosses. Tanks were fed 2.5 ($n = 5$), 7.5 ($n = 4$), 12.5 ($n = 4$) or 17.5 ($n = 5$) rotifers per mL of tank water, two times per day (930 h, 1630 h). All tanks received 2.5 rotifers per mL of

tank water at 2400 h. In each treatment, prey densities decreased with time after each feeding due to consumption by larvae and dilution from the flow-through water exchange. This 'pulsed' feeding method was chosen over a continually maintained feed density because pulsed feedings are more realistic for commercial aquaculture. After one week, we recorded survival and group dry weight of 30 larvae per tank.

Statistical analyses

Kruskal–Wallis tests and *post hoc* Tukey HSD were used to test for effects of feed density on total surviving biomass, survival and weight.

Results

Experiment 1

In the 960 and 1920-L tanks, the proportion of swimming (as opposed to drifting) larvae increased with tank size ($P < 0.01$, Fig. 3a) and light intensity ($P < 0.05$, Fig. 3a). The interaction between tank size and light intensity did not reach statistical significance ($P < 0.07$). The number of larvae seen at the surface was not affected by tank size, light intensity, or an interaction between tank size and light intensity ($P > 0.05$). The number of feeding strikes per larva was affected by tank size ($P < 0.05$, Fig. 3b), light intensity ($P < 0.05$, Fig. 3b), and an interaction between tank size and light intensity ($P < 0.05$). Feeding strikes ranged from 0 to 0.8 strikes per larva per 30 s in the 960-L tanks and from 0.41 to 1.13 strikes per larva per 30 s in the 1920-L tanks. The 960-L tanks appeared to have peak feeding strikes around 250–300 lux (range tested about 40–800 lux in both 960-L tanks), while the 1920-L tanks had peak feeding strikes at the lowest light intensities provided (66–126 lux).

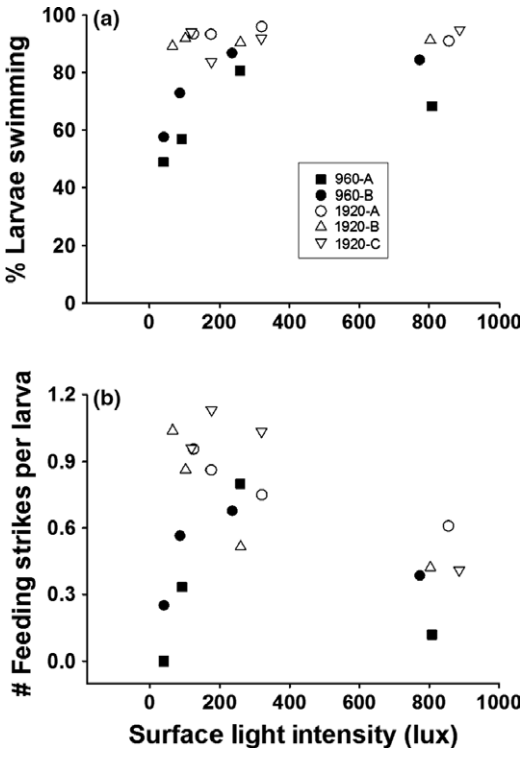

Figure 3 Swimming activity (a) and number of feeding strikes per larva per 30 s (b) in 960-L and 1920-L tanks as a function of light intensity.

Experiment 2

In the 37-L tanks, intermediate light intensities had greater total surviving biomass ($P < 0.05$, Fig. 4a) and greater weight (30 larvae weighed together, $P < 0.05$, Fig. 4b) than both the highest

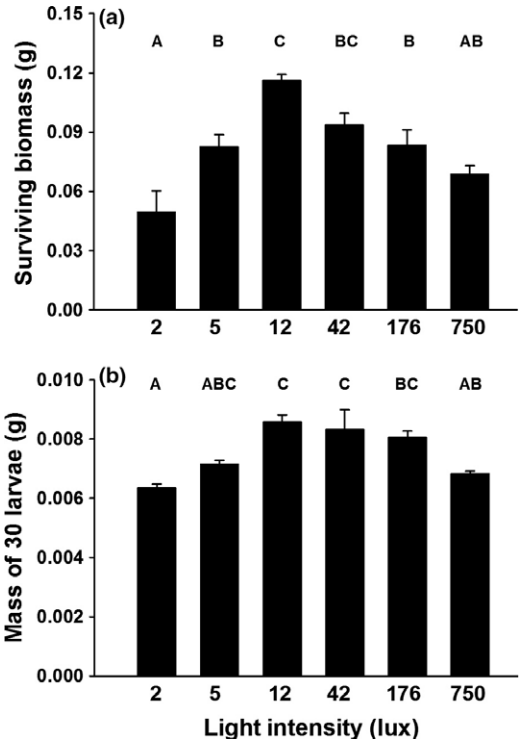

Figure 4 Total surviving biomass (a) and mass of 30 larvae (b) as a function of light intensity in 37-L tanks. Data are from trial 2, with $n = 3$ tanks per light intensity. Error bars show standard error. Different letters represent statistically significant differences.

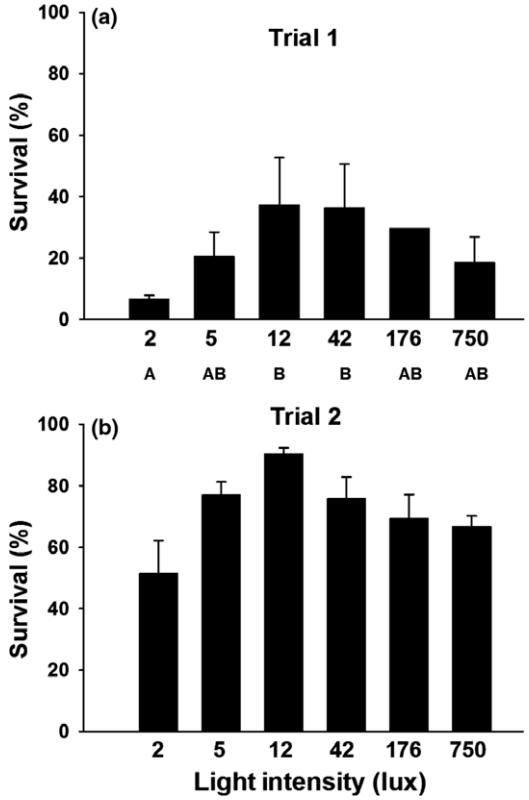

Figure 5 Survival as a function of light intensity in trials 1 (a, $n = 2$ tanks per light intensity, except for the 176 lux treatment which had $n = 1$) and 2 (b, $n = 3$ tanks per light intensity) in 37-L tanks. Error bars show standard error. Different letters represent statistically significant differences.

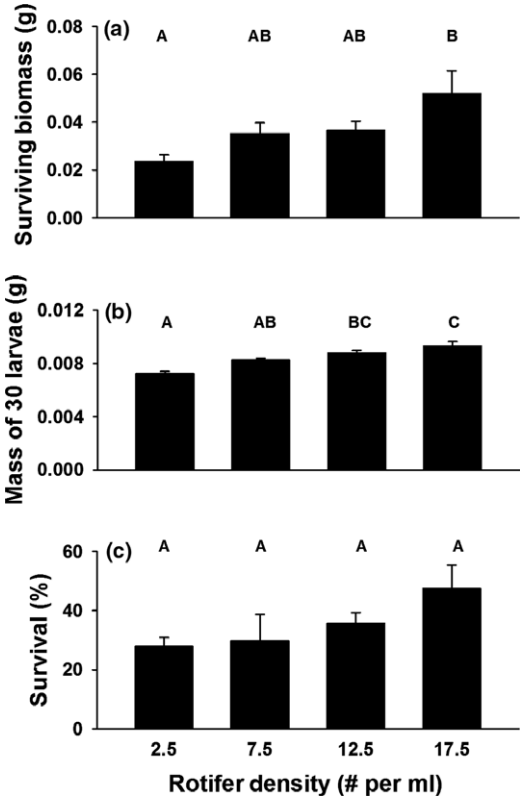

Figure 6 Total surviving biomass (a), mass of 30 larvae (b), and survival (c) as a function of feed density in 37-L tanks. For the 2.5, 7.5 12.5 and 17.5 rotifers per mL treatments, $n = 5$, 4, 4, and 5 respectively. Error bars show standard error. Different letters represent statistically significant differences.

and lowest light intensities. Survival also was significantly affected by light intensity ($P < 0.01$, Fig. 5), with intermediate intensities tending to increase survival. The percentage of larvae surviving the second trial was nearly three times greater than in the first trial ($P < 0.0001$, Fig. 5).

Experiment 3

In the 37-L tanks, total surviving biomass and weight per 30 larvae significantly increased with feed density ($P < 0.05$, Fig. 6a and $P < 0.01$, Fig. 6b respectively). Survival was not significantly affected by feed density ($P = 0.38$, Fig. 6c).

Discussion

The sablefish aquaculture industry is young, and many industry standards for hatchery rearing have not yet been established. These experiments help define sablefish rearing methods and show how growth and survival can be improved by optimizing light intensity, and further improved with increased feed densities.

Most marine fish hatcheries use light intensities between 1000 and 2000 lux (Planas & Cunha 1999). In most studies, higher light intensity has led to increased activity, feeding, growth and survival. Several studies have tested young larvae at a range of light intensities similar to, or encompassing, the range tested in this study. Toledo, Caberoy, Quinitio, Choresca and Nakagawa (2002) reared newly hatched grouper larvae (*Epinephelus coioides*) for six days under light intensities ranging from 0 to 700 lux, measured at the middle layer of 40-L aquaria. Feeding incidence and survival were greatest at the highest light intensities. Endogenous oil globules were also depleted most quickly at higher

light intensities, suggesting higher activity. Similarly, the larval sablefish in our study showed greater activity (more larvae swimming) under brighter light. Yoseda, Yamamoto, Asami, Chimura, Hashimoto and Kosaka (2008) reared leopard coral grouper (*Plectropomus leopardus*) from zero to six days after hatch at light intensities ranging from 0 to 3000 lux, measured at the water surface, and found greater feeding, growth and a tendency for greater survival at higher light intensities. Stuart and Drawbridge (2011) reared California yellowtail (*Seriola lalandi*) from two to 16 days post hatch at 360, 1675, and 14 850 lux, measured at the water surface, and found the highest growth and survival at the brightest two treatments. Nicolaisen, Cuny and Bolla (2014) tested the foraging success of 5-, 10-, 15- and 20-day-old Atlantic cod (*Gadus morhua*) under light intensities of 100, 650 and 1200 lux at the water surface. In those Atlantic cod, more intense light led to increased foraging success, particularly with older larvae and with white tank bottoms.

In sablefish and other species, improved feeding at lower light intensities may reflect adaptations to their natural environments. Visual system evolution is driven by natural light environments, which are influenced by variables such as larval depth, water turbidity, spawning season and time of day (Puvanendran & Brown 1998; Vollset, Folkvord & Browman 2011). Newly hatched halibut larvae (*Hippoglossus hippoglossus*) showed equal survival under brighter light and developed more mouth deformities if exposed to any light during yolk absorption (0, 3, 30 and 300 lux tested; Bolla & Holmefjord 1988). Sea bass larvae (*Dicentrarchus labrax*) grew larger but showed poorer survival under bright light, likely because bright light is damaging before total pigmentation (bright treatment was 1400–3500 lux at the surface; low-intensity treatment was 150–800 lux; Barahona-Fernandes 1979). The preference of newly hatched sea bass for low light early in life may reflect a tendency to remain near the sea bottom soon after hatching (Barahona-Fernandes 1979). Puvanendran and Brown (1998) reared Atlantic cod larvae (*Gadus morhua*) under 8.5 and 680 lux at the water surface and found greater growth at 8.5 lux in the Scotian Shelf population but greater growth at 680 lux in the Northeast Grand Banks population. They suggest that these population differences may be adaptive responses to their natural environments; the Scotian Shelf population spawns during the late fall and early winter so their larvae typically experience less intense light than the spring- and summer-spawning Northeast Grand Banks population. Our experiments with sablefish larvae put them in this minority of species that have higher growth or survival with light intensity below 800 lux. Larval sablefish exhibited more feeding strikes and better surviving biomass at low light intensities. Like Scotian Shelf Atlantic cod, sablefish spawn during the winter (January–March). Developed larvae have been collected at surface waters in April off British Columbia (Shaw & McFarlane 1997) or May off Oregon and Washington (Boehlert & Yoklavich 1985), but the depth of newly hatched sablefish is unknown. For new aquaculture species, natural spawning season timing and larval depth distributions may inform pilot culture methods for light intensities.

While larvae in all tank types preferred light intensities less than 800 lux, there were some finer scale differences among tank types that might be related to variables such as tank dimensions, larval age and differences in types of response variables. The 37-L tanks appeared to have a dimmer optimal light intensity than the 960-L and 1920-L tanks. Total surviving biomass in 37-L tanks was greater at 12 lux than at 176 or 750 lux, while feeding strikes were more frequent in 960-L and 1920-L tanks at 100–300 lux than at 800 lux. Tank depth differences may have led to differences in the amount of light reflecting upwards from the bottom (depth: 37 L – 36.5 cm, 960 L – 152 cm, 1920 L – 121 cm). All tanks had black walls and white bottoms. As light intensity diminishes with depth (Table 1) and white surfaces reflect light (Naas *et al.* 1996), more light should reach the bottoms and reflect upwards in shallower 37-L tanks. There were also significant or marginally non-significant interactions between light intensity and tank type (960 L, 152 cm deep versus 1920 L, 121 cm deep) for feeding behaviour and activity level. Feeding and activity levels declined at the lowest light intensities in the 152-cm-deep tanks but not in the 121-cm-deep tanks, perhaps suggesting that in the deeper tanks too little light reached the bottom to be reflected at sufficient intensities. Tank diameter differences may have also played a role (diameter: 37 L – 37 cm, 960 L – 104 cm, 1920 L – 152 cm). Larvae in smaller diameter tanks may have experienced more light and scatter reflecting from the sides, which is an important direction because the larvae look

horizontally to feed. Differences in tank volumes and tank dimensions also likely affect survival (Cook *et al.* 2015). Survival to weaning is typically higher in the 1920-L tanks than in the 960-L tanks (1920 L – 21.1%, 960 L – 13.2%, Cook *et al.* 2015). We have not quantified survival to weaning in the 37-L tanks, but survival to two weeks after first feeding appears significantly lower in 37-L tanks than in 960-L or 1920-L tanks (J. Lee, M. Cook, personal observations).

Larval age may have also had effects on optimal light levels. Larval ages differed between experiments in 37-L tanks (first feed to 7 days) and the larger tanks (10–11 days after first feed). Light intensity requirements (Villamizar, Blanco-Vives, Migaud, Davie, Carboni & Sanchez-Vazquez 2011) and spectral sensitivity of larval sablefish may undergo significant changes during early ontogeny. Preliminary measurements of the visual pigments of first-feeding sablefish larvae found that they possess two predominant cone types, UV-sensitive (~385 nm) and blue-sensitive (~480 nm) single cones (L. Britt, unpublished data). Within five days of first feeding, rudimentary rods (~500 nm) were also observed. This is a highly unusual spectral sensitivity range (Britt, Loew & McFarland 2001) and is currently being confirmed.

Response variables also differed between 37-L tanks and the larger tanks. The 37-L tanks were used to measure effects of light on growth and survival, which is a sum of all energy input and output, whereas the larger tanks were used to measure activity and feeding behaviour, which are just components of the total energy input and output. Determining the causes of these finer scale differences in results among tanks will likely be important to further optimize lighting conditions for a range of tank types and sizes.

The light intensity experiment in the 37-L tanks showed threefold differences in survival between the two replicate trials (Fig. 5). Thus, while comparisons among treatments can be made within trials, it is not possible to directly compare absolute survival rates between experiments (for example, between light intensity and prey density experiments). The survival rates in the two replicate trials represent the low and high ends of the usual range in survival rates that we typically have observed in sablefish experiments in our laboratory. One of our current areas of focus includes trying to uncover the mechanisms responsible for this kind of seemingly random variation in survival.

Optimal light intensity and coloration improve prey encounter rates by maximizing the larval visual range and prey contrast against the prevailing background illumination, but higher prey density can further increase prey encounter rates. The prey density study utilized the optimal light intensity identified in the light intensity study and showed that higher prey density can lead to increases in surviving biomass and growth. We expected growth and survival to increase with feed density, up to a plateau where consumption became limited by time spent capturing and digesting prey. However, the lack of a plateau indicates that search time was important throughout the range of feed density treatments (2.5–17.5 rotifers per mL of tank water). Optimal feed density may differ as a function of tank flushing rates (flow rates), which can differ among hatcheries. These rates should be taken into account when extrapolating these results to other hatcheries. Results might also differ if feed densities are kept constant through time, with feed being added continuously to replace feed that has been flushed out with flow-through water or consumed by larvae. We did not keep feed densities constant through time because our goal was to refine rearing methods for commercial aquaculture, and constantly maintained feed densities are currently impractical for commercial aquaculture facilities because of labour constraints and feed enrichment schedules.

These data show how variations in lighting and feed densities can impact behaviour, growth and survival, and help refine larval sablefish rearing methods. Future work should address variables such as larval age, photoperiod, feed frequency, and interactions among variables including light intensity and feed density (Martin, Wahl & Czesny 2012; Nicolaisen *et al.* 2014).

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