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**Life history of the symbiotically luminous cardinalfish**

***Siphamia tubifer* (Perciformes: Apogonidae)**

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Running headline: LIFE HISTORY OF *SIPHAMIA TUBIFER*

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Characteristics of the life history of the coral reef-dwelling cardinalfish *Siphamia tubifer*, from Okinawa, Japan, were defined. A paternal mouthbrooder, *S. tubifer* is unusual in forming a bioluminescent symbiosis with *Photobacterium mandapamensis*. The examined *S. tubifer* ( $n = 1273$ ) ranged in size from 9.5 to 43.5 mm standard length ( $L_S$ ) and the minimum size at sexual maturity was 22 mm  $L_S$ . The number of *S. tubifer* associated during the day among the spines of host urchins was  $22.9 \pm 16.1$  (mean  $\pm$  S.D.; *Diadema setosum*) and  $3.6 \pm 3.2$  (*Echinothrix calamaris*). Diet consisted primarily of crustacean zooplankton. Batch fecundity (number of eggs;  $F_B$ ) was related to  $L_S$  by the equations: males (fertilized eggs)  $F_B = 27.5L_S - 189.46$ ; females (eggs)  $F_B = 31.3L_S - 392.63$ . Individual mass ( $M$ ; g) as a function of  $L_S$  was described by the equation:  $M = 9.74 \times 10^{-5} L_S^{2.68}$ . Growth, determined from otolith microstructure analysis, was described with the von Bertalanffy growth function with the following coefficients:  $L = 40.8$  mm  $L_S$ ,  $K = 0.026$  day $^{-1}$  and  $t_0 = 23.25$  days. Planktonic larval duration was estimated to be 30 days. The age of the oldest examined individual was 240 days. The light organ of *S. tubifer*, which harbours the symbiotic population of *P. mandapamensis*, increased linearly in diameter as *S. tubifer*  $L_S$  increased and the bacterial population increased logarithmically with *S. tubifer*  $L_S$ . These characteristics indicate that once settled, *S. tubifer* grows quickly, reproduces early and typically survives much less than a year in Okinawa. These characteristics are generally similar to other small reef fishes but they indicate that *S. tubifer* experiences higher mortality.

Key words: apogonids; bioluminescence; diet; growth; reproduction; symbiosis.

## INTRODUCTION

Cardinalfishes (Perciformes: Apogonidae) are common on coral reefs worldwide and are often among the most abundant fish family present in a community (Bellwood, 1996).

Cardinalfishes are paternal mouthbrooders (Vagelli, 2011), active foragers at night (Marnane & Bellwood, 2002), and seek shelter during the day, often in high densities with other reef structures, including reef-dwelling invertebrates (Gardiner & Jones, 2005, 2010). As highly abundant, small-bodied fishes that often exhibit fidelity to a reef site, cardinalfishes probably play an important role as prey for larger, predatory fishes and as planktivores that help recycle nutrients within the reef community (Marnane, 2000; Marnane & Bellwood, 2002).

Despite the diversity and ecological importance of cardinalfishes, comprehensive knowledge of the life history of individual species remains limited, and key life-history traits critical for population success, such as growth and reproductive rates, are not widely documented. Nearly 350 species of cardinalfishes have been identified (Eschmeyer & Fong, 2015), yet the growth rates of only six species have been described in detail (Kume *et al.*, 1998; Okuda *et al.*, 1998; Kume *et al.*, 2003; Longenecker & Langston, 2006; Raventós, 2007; Wu, 2009; Ndobe *et al.*, 2013; Kingsford *et al.*, 2014). Many studies to date have emphasized the reproductive biology of cardinalfishes (Kuwamura, 1985; Vagelli, 1999; Kume *et al.*, 2000b; Okuda, 2001; Fishelson & Gon, 2008), whereas a few studies have described larval growth and the pelagic larval duration (PLD) of some cardinalfish species

(Brothers *et al.*, 1983; Ishihara & Tachihara, 2011; Kingsford *et al.*, 2014; Leis *et al.*, 2015) as well as the diets and feeding ecologies of others (Chave, 1978; Marnane & Bellwood, 2002; Barnett *et al.*, 2006). Additionally, certain aspects of the behavioural ecology, *e.g.* sociality, site fidelity and homing behaviour, of a few species have been recently described (Marnane, 2000; Gardiner & Jones, 2005; Kolm *et al.*, 2005; Døving *et al.*, 2006; Gardiner & Jones, 2010; Gould *et al.*, 2014; Rueger *et al.*, 2014).

Life-history information is particularly sparse for bioluminescent cardinalfishes. There are three genera with autogenously luminous species, *Archamia*, *Jaydia* and *Rhabdamia*, and one bacterially luminous genus, *Siphamia* (Thacker & Roje, 2009). The sea urchin cardinalfish *Siphamia tubifer* Weber 1909 (Gon & Allen, 2012), formerly classified as *Siphamia versicolor* (Smith & Radcliffe, in Radcliffe, 1911; Tominaga, 1964), is possibly the most widespread species of luminous cardinalfishes. Some characteristics of the ecology and behaviour of *S. tubifer* have been documented recently (Gould *et al.*, 2014, 2015), and symbiosis of *S. tubifer* with the luminous bacterium *Photobacterium mandapamensis* has been the subject of several studies (Leis & Bullock, 1986; Wada *et al.*, 2006; Kaeding *et al.*, 2007; Dunlap *et al.*, 2009, 2012; Dunlap & Nakamura, 2011). Key aspects of the life history of *S. tubifer*, including diet, reproduction and growth, however, remain largely undescribed. There is a general lack of detailed life-history knowledge for the > 450 species of bacterially luminous fishes found worldwide (Nelson, 2006; Froese & Pauly, 2015). Therefore, the general goal of this study was to describe in detail, aspects of the life history of *S. tubifer* in order to provide a foundation for understanding the biology of *S. tubifer* for future research and to provide an additional perspective on the biology of both reef-dwelling cardinalfishes

and bacterially luminous fishes. The specific aims were to define the body size, length to mass ratio, size distribution, aggregation size, diversity of diet, reproduction, growth and symbiont population growth in host light organs of *S. tubifer*.

## MATERIALS AND METHODS

### FIELD COLLECTION

Juvenile and adult *S. tubifer* were collected from reefs at various locations in Okinawa, Japan (26.5° N; 128° E), during the summer months (June-August) of 2011 through 2014. *Siphamia tubifer* were collected with their host sea urchin with scuba using a gaff hook and a 20 l bucket; the resident group of *S. tubifer* remained with their host urchin as it was gently guided into a bucket using the hook. After collection, urchins were returned to their site of capture. The protocols used here for capture, care and handling of *S. tubifer* were approved by the University of Michigan's University Committee on Use and Care of Animals, and they conform to the University of the Ryukyus' Guide for Care and Use of Laboratory Animals (Dobutsu Jikken Kisoku, version 19.6.26).

The standard length ( $L_S$ ) of each *S. tubifer* was measured to the nearest 0.5 mm using calipers and the total wet mass ( $M$ ; g) of several individual *S. tubifer* was measured to the nearest 0.001 g. The  $L_S$  to  $M$  relationship was estimated using the function:  $M = aL_S^b$ , where  $a$  is a constant and  $b$  is the growth exponent (LeCren, 1951). In 2013, the numbers of *S. tubifer* associated with an individual host sea urchin, either *Diadema setosum* or *Echinothrix*

*calamaris*, were recorded for analysis of aggregation size of *S. tubifer* in association with each urchin species at two reefs with a high abundance of both urchins near Sesoko Station, the University of the Ryukyu's Tropical Biosphere Research Centre (26°38'N; 127°52'E).

## DIET

The nocturnal diet of *S. tubifer* was characterized for 27 individuals captured together at dawn immediately upon their return to their host urchin (*Diadema setosum*) after nocturnal foraging (Gould *et al.*, 2014). *Siphania tubifer* stomachs were removed and preserved in 10% buffered-formalin in sea water for gut content analysis. The prey items present in each stomach were examined using a stereomicroscope (Leica MZ 12.5; [www.leica-microsystems.com](http://www.leica-microsystems.com)) and identified to the best possible taxonomic classification level. The percentage of empty stomachs examined, or vacuity index ( $I_V$ ), was calculated as well as the percentage composition of each prey category for each *S. tubifer* with stomach contents. The mean percentage composition of each prey type was compared between small, reproductively immature *S. tubifer* and large, reproductively mature *S. tubifer*. The percentage occurrence (presence or absence) of each prey type among all individuals in each size class was also determined.

## REPRODUCTION

To describe the reproductive potential of *S. tubifer*, the sex of mature *S. tubifer* was determined by gonad examination, and the total number of fertilized eggs in the mouths of

brooding males and mature eggs within the body of gravid females were counted using a stereomicroscope (Leica MZ 12.5). Only the eggs from females with highly developed oocytes were counted, as they were easily separated from one another and individually sorted. The relationship between *S. tubifer*  $L_S$  and batch fecundity ( $F_B$ ), the number of eggs and fertilized eggs per clutch for gravid females and brooding males, respectively, was fitted with a linear regression. To test for a difference between batch fecundity of females and males, an ANCOVA was performed in R version 3.1.1 (R Core Team; [www.r-project.org](http://www.r-project.org)) with sex as the factor and *S. tubifer*  $L_S$  as the covariate. Five fertilized eggs per brood were also selected at random from 35 male *S. tubifer* and the egg diameters along two perpendicular axes (one slightly longer than the other) were measured to the nearest 0.05 mm using a stage micrometer and stereomicroscope (Leica MZ 12.5); the mean diameter of each perpendicular axis was calculated for each of the 35 broods examined.

## GROWTH

Pairs of sagittal otoliths from individual *S. tubifer* were removed, cleaned and stored dry for microstructure analysis. The diameters of the right sagittae from several specimens were measured using a dissecting microscope (Zeiss SteREO Discovery.V8; [www.zeiss.com](http://www.zeiss.com)) equipped with a digital camera (Zeiss AxioCam MRc). Images were taken of whole otoliths [Fig. 1(a)], and the longest axis through the primordium from one margin edge to the other was measured to the nearest 0.01 mm using Axio Vision 4 software (Zeiss). The percentage of *S. tubifer*  $L_S$  was also calculated for each otolith diameter.

To estimate *S. tubifer* age, individual otoliths were mounted and attached to glass slides with KrazyGlue (Elmer's Products, Inc.; www.elmers.com) and ground to their transverse mid-plane using 2000 grit wet/dry polishing paper until daily growth bands became visible. Both sides of larger otoliths were ground to clearly expose bands; however, smaller otoliths were ground only on one side if growth bands became clearly visible. Images of each cross-section were taken with transmitted light under a compound microscope (Nikon Eclipse E600; www.nikon.com) equipped with a SPOT 2 Slider (1.4.0) digital camera, and the total number of daily growth bands along a continuous radial transect, if possible, was counted twice, each time by a different observer for each image, using Adobe Photoshop (CS6 Extended; www.adobe.com). First daily increment formation typically occurs at or close to hatching in reef fishes (Thorrold & Hare, 2002); therefore growth bands were counted from the first visible band after the hatch mark, a distinct, dark circle surrounding the primordium, to the outer margin of each otolith. The increments between growth bands were also measured for several individuals ( $n = 10$ ) to look for a change in increment width as an indication of the timing of settlement out of the plankton (Victor, 1986; Kingsford *et al.*, 2011).

The average number of bands counted for each otolith by both observers was used as the final value for the age of the *S. tubifer*. If the two counts differed by  $> 10\%$ , a third count was made and the average of the closest two counts was used as the *S. tubifer*'s age. If the third count was not within 10% of the first two counts, the otolith was not included in the analysis. Growth was described by the von Bertalanffy growth function:  $L_t = L_{\infty}[1 - e^{-K(t-t_0)}]$

where  $L_t$  is the  $L_S$  at time  $t$  (days),  $L_\infty$  is the  $L_S$  at which mean asymptotic growth is reached,  $K$  is the growth coefficient ( $\text{days}^{-1}$ ), and  $t_0$  is the theoretical age (days) at which fish length is zero. The function was fitted to individual length-at-age data for *S. tubifer* using the package ‘fishmethods’ version 1.7-0 (Nelson, 2014) in R version 3.1.1 (R Core Team). Akaike information criterion (AIC) scores were determined and used for selection of the von Bertalanffy growth model over a linear growth model.

Daily growth bands were verified for sagittal otoliths of *S. tubifer* using a tetracycline immersion method (Kingsford *et al.*, 2014) at Sesoko Station in June 2013. Adult *S. tubifer* ( $n = 50$ , ranging in  $L_S$  from 23 to 41.5 mm) were immersed in a  $0.25 \text{ g l}^{-1}$  tetracycline solution in buffered sea water with aeration for 18 h and maintained in aquaria under natural light conditions ( $26^\circ\text{N}$ ) with flowing sea water for an additional 5, 10 or 15 days after immersion. While in aquaria, *S. tubifer* were fed nightly with an excess of wild-caught live zooplankton, collected with a  $53 \mu\text{m}$  zooplankton net using a spotlight at dusk from the pier adjacent to their home reef, to simulate the natural diet and timing of foraging. Following each post-immersion period, the sagittae of the randomly selected and sacrificed individuals were immediately removed and stored dry in the dark until processing. Otolith cross-sections were examined under a compound microscope (Nikon Eclipse E600) with fluorescent light for tetracycline marks (Odense & Logan, 1974); if a fluorescent band was visible, the otolith was photographed in the same position under both transmitted and fluorescent light [Fig. 1(b)]. The number of bands present between the fluorescent mark and the otolith margin for each otolith with a visible tetracycline mark was counted without knowledge of the number of days post-immersion that the *S. tubifer* was sacrificed. The band counts were then compared

to the number of days post-treatment for each *S. tubifer* and averaged within each treatment group.

## LIGHT ORGAN AND SYMBIONT POPULATION GROWTH

Light organs of *S. tubifer* were dissected out and measured on the longer, anterior to posterior axis to the nearest 0.1 mm using a stereomicroscope (Leica MZ 12.5) fitted with an ocular micrometer. To quantify bacterial population sizes, light organs from *S. tubifer* of different  $L_S$  were aseptically dissected and individually homogenized in 0.5 ml of filter-sterilised (0.2 mm) 70% sea water 25 mM HEPES buffer (pH 7.25) in ethanol-sterilised, hand-held tissue grinders (Kaeding *et al.*, 2007). The homogenates were then serially diluted 1:100 and again 1:100 in filter-sterilised, buffered 70% artificial sea water, and 25  $\mu$ l aliquots of the second dilution were spread onto plates of a sea water-based nutrient agar medium (Kaeding *et al.*, 2007), which contained per litre: 10 g tryptone, 5 g yeast extract, 700 ml sea water, 300 ml de-ionized water and 15 g of agar. The plates were then incubated at room temperature (25 – 29° C) for 12 to 18 h to allow the formation of bacterial colonies. The bacterial colonies were counted in the light to quantify the number of colonies, and in the dark in a photographic darkroom to confirm that all colonies were luminous and had the characteristic appearance of the symbiont, *P. mandapamensis*. Light organ population sizes were calculated from colony counts times the dilution factor used. Population sizes were  $\log_{10}$ -transformed, and the relationship with *S. tubifer*  $L_S$  was fitted linearly.

## RESULTS

## SIZE

The  $L_S$  of *S. tubifer* collected from reefs in Okinawa over the 4 year study period ( $n = 1273$ ) ranged from 9.5 to 43.5 mm; 12% of the *S. tubifer* observed were  $> 32$  mm  $L_S$  (Fig. 2). Brooding males ( $n = 95$ ) ranged in  $L_S$  from 22 to 43.5 mm (Fig. 2). The  $M$  of *S. tubifer* increased as a curvilinear function with *S. tubifer*  $L_S$  (Fig. 3). The mean  $\pm$  S.D.  $M$  of all *S. tubifer* weighed was  $0.780 \pm 0.540$  g, with minimum and maximum masses of 0.043 and 2.300 g, corresponding to *S. tubifer* that were 11.0 and 42.5 mm  $L_S$ , respectively.

The numbers of *S. tubifer* associated in groups with individual host urchins varied between the two urchin species; the mean  $\pm$  S.D. number of *S. tubifer* associated with *D. setosum* was  $22.9 \pm 16.1$ , whereas the mean  $\pm$  S.D. number associated with *E. calamaris* was  $3.6 \pm 3.2$  (Fig. 4). Approximately 35% of the *E. calamaris* examined ( $n = 69$ ) were occupied by one *S. tubifer*, whereas only 6% of *D. setosum* ( $n = 36$ ) were occupied by a single *S. tubifer* (Fig. 4). Moreover, the largest group of *S. tubifer* observed with an *E. calamaris* urchin contained 15 individuals, less than the mean group size associated with *D. setosum*. The largest group of *S. tubifer* associated with a *D. setosum* urchin consisted of 75 individuals.

## DIET

The general nocturnal diet of *S. tubifer* was diverse, consisting primarily of a variety of crustaceans and other zooplankton, and gut contents varied between individuals collected together from the same urchin (Table I). The only empty stomachs observed ( $I_V = 7.4\%$ ) were

from the two brooding males collected, confirming that *S. tubifer* does not forage while brooding (Gould *et al.*, 2014). Brooding males were excluded from the diet analysis. On one occasion, 31 eggs (0.5 mm in diameter) of an unknown fish species were present in the stomach of a large, non-brooding *S. tubifer* (26 mm  $L_S$ ) in addition to other prey items. The diets of small and large *S. tubifer* differed somewhat, although both amphipods and small decapod shrimp were common prey items for both size classes; amphipods made up *c.* 18 and 10% of the diets of small and large *S. tubifer*, whereas decapod shrimps composed a mean of over 30% of the diet of all *S. tubifer* examined (Table I). Several prey items were present in over half of the small individuals examined, including amphipods, decapod crab zoea and decapod shrimps. Over half of the large *S. tubifer* also consumed decapod shrimps, however, nearly half of this size class also consumed mysid shrimps and small teleost larvae, which were unidentifiable due to digestive state. Copepods were observed in the stomachs of both size classes but made up a larger percentage of the diets of smaller *S. tubifer* (Table I).

## REPRODUCTION

Total numbers of fertilized eggs in the buccal cavity of brooding *S. tubifer* males ranged from 415 to 838 with a mean  $\pm$  S.D. of  $650 \pm 146$ , and the numbers increased linearly with  $L_S$  (Fig. 5). Similarly, the total number of eggs in ovaries of gravid females increased linearly with  $L_S$ , ranging from 440 to 838 total eggs, and the mean  $\pm$  S.D. was very similar to that for males,  $678 \pm 164$ . The relationship between  $F_B$  and  $L_S$  was not significantly different between males and females (ANCOVA,  $F_{2,65} = 2.7$ ,  $P > 0.05$ ; Fig. 5). Fertilized eggs in the mouths of male *S. tubifer* were nearly round, with one axis slightly longer than its

perpendicular axis; the mean  $\pm$  S.D. long axis diameter was  $0.87 \pm 0.07$  mm, whereas the shorter axis was  $0.80 \pm 0.07$  mm. The ranges in diameter were 0.65 - 1.00 mm and 0.70 - 1.20 mm for the short and long axes, respectively.

## GROWTH

Sagittal otolith diameter ( $O_L$ ) increased linearly with *S. tubifer*  $L_S$  ( $L_S = 15.0O_L - 1.16$ ,  $r^2 = 0.97$ ) and ranged from 0.83 mm to 3.07 mm (Fig. 6). The mean  $\pm$  S.D.  $O_L$  s as a percentage of *S. tubifer*  $L_S$  was  $7.05 \pm 0.44\%$ . From counts of otolith daily increments, the growth of *S. tubifer* was described by the von Bertalanffy growth model (VBGM) (Fig. 6), which indicated that asymptotic growth is reached at 40.8 mm  $L_S$ . The relationship between  $\ln(L - L_\infty)$  and apparent age of *S. tubifer* was linear ( $r^2 = 0.82$ ) and validated the use of the VBGM (Everhart & Youngs, 1981), as did a comparison of AIC scores between a linear model and the VBGM; the AIC score for the VBGM was considerably lower ( $\Delta$  AIC = 145). Based on this growth curve, the age at first reproduction of *S. tubifer* is *c.* 57.5 days at 22 mm  $L_S$ , the smallest size observed of reproductively mature *S. tubifer*. Furthermore, the age of the oldest individual examined was estimated to be 240 days at 43 mm  $L_S$ . Settlement marks were not evident in *S. tubifer* otoliths and there was no observable pattern in increment width between growth bands that would indicate the timing of settlement; increment widths varied overall from 4.8 – 19.5  $\mu\text{m}$  (mean  $\pm$  S.D.  $13.8 \pm 3.2 \mu\text{m}$ ). The youngest *S. tubifer* analysed, however, was 31 days old (11.5 mm  $L_S$ ), which was close to the smallest size of *S. tubifer* collected with an urchin (Fig. 2); therefore the PLD for *S. tubifer* in Okinawa is estimated to be *c.* 30 days.

The tetracycline immersion method confirmed that the growth bands used for ageing represented daily growth increments of *S. tubifer*. Sagittal otoliths of 22% of the chemically treated *S. tubifer* showed clear incorporation of tetracycline into their otolith microstructure, visible as a fluorescent band under UV light [Fig. 1(b)]. The number of bands between the fluorescent mark and the otolith margin of these otoliths corresponded with the number of days post-immersion for these individuals (Table II).

#### LIGHT ORGAN AND SYMBIONT POPULATION GROWTH

Light organs of *S. tubifer* increased linearly in diameter as *S. tubifer*  $L_S$  increased with no sign of asymptotic growth [ $r^2 = 0.82$ , Fig. 7(a)]. The smallest light organ measured was 0.8 mm in diameter (11.0 mm  $L_S$ ), and the two largest light organs were both 2.9 mm in diameter (36.0 and 37.0 mm  $L_S$ ). The population sizes of *P. mandapamensis* in light organs also increased with *S. tubifer*  $L_S$  [ $r^2 = 0.62$ , Fig. 7(b)], increasing from  $7.0 \times 10^6$  (13.8 mm  $L_S$ ) to  $8.7 \times 10^7$  (38.2 mm  $L_S$ ) cells.

#### DISCUSSION

Among cardinalfishes, *S. tubifer* is unusual for its symbiosis with the luminous bacterium *Photobacterium mandapamensis*. This study provides additional evidence of the distinct biology of *S. tubifer* and also highlights some biological similarities to other apogonids, including a diverse, carnivorous diet and large group aggregation sizes. In

particular, the results presented here indicate that *S. tubifer* is the shortest-lived cardinalfish studied to date, which in addition to a high natural mortality rate, might result from high predation pressure, as direct predation on *S. tubifer* by other reef fishes has been observed at the study site (Gould *et al.*, 2014). As a consequence, predation might have played a role in shaping the cryptic behaviour of *S. tubifer* as a small, bioluminescent coral reef fish that seeks refuge among urchin spines during the day and uses ventral luminescence, potentially for counter-shading, while foraging at night (Dunlap & Nakamura, 2011).

Within the cardinalfish family, there is an overall positive, linear relationship between maximum species size and longevity (Marnane, 2001). This relationship holds true for small species such as Doederlein's cardinalfish *Ostorhinchus doederleini* (Jordan & Snyder 1901) and the rubyspot cardinalfish *Ostorhinchus rubrimacula* (Randall & Kulbicki 1998), the life histories of which have both been recently described (Table III). Results of this study are similar to that of *O. rubrimacula*, suggesting that *S. tubifer* is also short-lived in Okinawa (<2 to 3 year), and support the positive relationship between maximum body size and longevity of apogonids. In addition, the size distributions reported for other *Siphamia* species are similar to that of *S. tubifer*; the coral siphonfish *Siphamia corallicola* Allen 1993 and Jebb's siphonfish *Siphamia jebbi* Allen 1993 range in  $L_S$  from 10.7 to 30.5 mm ( $n = 55$ ) and 11.7 to 24.8 ( $n = 39$ ), respectively (Allen, 1993). It should be noted, however, that a larger maximum body size (7.0 cm total length,  $L_T$ ) of *S. tubifer* was reported from Tahiti on FishBase (Froese & Pauly, 2015), which indicates that *S. tubifer* has the potential to live longer than observed in this study.

Aggregation sizes vary between different species of cardinalfishes and can range from solitary or paired individuals to hundreds of individuals. Gardiner & Jones (2010) determined that the mean group size of the five-lined cardinalfish *Cheilodipterus quinquelineatus* Cuvier 1828 was 13 with over half of observed groups consisting of only one to six individuals, whereas longspine cardinalfish *Zoramia leptacantha* (Bleeker 1856) groups were much larger, averaging 98 individuals and containing as many as 700 individuals. The number of individuals per aggregation reported for *S. jebbi* was between 20 and 40 individuals in association with pocilloporid coral heads (Allen, 1993). This aggregation size is similar to the number of *S. tubifer* reported here in association with *D. setosum*, but the group size associated with *E. calamaris* was much lower, probably due to the shorter spines of *E. calamaris*, which cannot physically accommodate or protect a large number of individuals. The group size of *S. tubifer* associated with *E. calamaris* was similar to the aggregation size reported for *Siphamia* spp. among the spines of the crown-of-thorns sea star *Acanthaster planci* (two to 18 individuals per group, mean = 6.2) (Stier *et al.*, 2009), which also have shorter spines than *D. setosum*. Conversely, the numbers of silver siphonfish *Siphamia argentea* Lachner 1953 associated with the sea urchin *Astropyga radiata* in Madagascar were reported to be so large that the sea urchin could not accommodate all of the individuals; *S. argentea* therefore formed a dense aggregation in the form of an urchin directly above the urchin itself (Fricke, 1970).

Despite the use of ventral luminescence while foraging, the diet of *S. tubifer* was similar to that reported for non-luminous cardinalfishes. Most apogonids are nocturnal predators and feed primarily on benthic invertebrates and zooplankton; their diet is diverse,

yet often dominated by crustaceans (Hiatt & Strasburg, 1960; Allen, 1993; Marnane & Bellwood, 2002; Longenecker & Langston, 2006). Similar to the findings reported here, the diet of *S. tubifer* (*S. permutata*) from the Red Sea was reported to consist of copepods, gastropod veligers, worm chaeta, stomatopod larvae, benthic amphipods and juvenile shrimps (Fishelson *et al.*, 2005). All brooding males examined in this study had empty stomachs, and therefore, apparently do not forage during the incubation period. One non-brooding individual had several eggs in its stomach, but the eggs were smaller in diameter than *S. tubifer* eggs; filial cannibalism, as reported for other cardinalfishes (Okuda & Yanagisawa, 1996; Okuda, 1999; Kume *et al.*, 2000a) was not observed in this study. Although daytime feeding was not examined, *S. tubifer* may consume small zooplankton prey throughout the day while sheltered among its host urchin's spines (Magnus, 1967, Tamura, 1982). Overall, *Siphamia* spp., like most cardinalfishes, have a generalist carnivore diet and forage nocturnally on a diverse array of benthic zooplankton prey, especially decapod shrimps.

Mouth-brooding is one of the most effective ways of protecting offspring under high predation pressure (Oppenheimer, 1970), and is therefore a successful reproductive strategy for the relatively small-bodied family of cardinalfishes. Within the family, however, brood sizes carried by males vary widely, from as low as 40 to tens of thousands of eggs, as do egg diameters (Vagelli, 2011), and there is no indication that brood or egg size vary in relation to fish body size. Smaller cardinalfish species, including *Siphamia* spp., however, generally have ovaries that are relatively large compared to their body size and spawn fewer, larger eggs than do larger species (Fishelson & Gon, 2008). Within *Siphamia*, a physically small cardinalfish genus, brood sizes have been reported as low as 162 eggs for *S. corallicola* (25.0

mm  $L_S$ ) (Allen, 1993) and up to 600 for the crown-of thorns cardinalfish *Siphamia fuscolineata* Lachner 1953 (27.7 mm  $L_S$ ) (Vagelli, 2011); both instances were reported for a single brooding male. The mean number of eggs per brood for *S. tubifer* was similar to that reported for *S. fuscolineata*, however, the largest brood in this study contained over 800 eggs. The number of eggs previously reported in the ovaries of *S. tubifer* (26 mm  $L_S$ ) (Fishelson & Gon, 2008) corresponds with the lower range of total eggs counted in gravid *S. tubifer* females in this study. There was little to no difference between the total number of fertilized eggs in broods carried by male *S. tubifer* and eggs in female gonads, indicating that few, if any, eggs are lost in the process of fertilization and transfer to the male.

Egg diameters reported for other *Siphamia* spp. are similar to those reported here and elsewhere (Tominaga, 1964); fertilized eggs of *S. corallicola* and *S. fuscolineata* were between 0.95 and 1.0 mm and 0.7 and 0.8 mm in diameter (Allen, 1993; Vagelli, 2011), respectively. Egg diameters in female ovaries of *S. tubifer* (*S. permutata*) and the pink-breasted siphonfish *Siphamia roseigaster* (Ramsay & Ogilby 1887), however, were 1.2 and 1.3 mm in diameter (Fishelson & Gon, 2008), both larger than the maximum diameter of fertilized eggs observed in the mouths of *S. tubifer* males in this study. Overall, *Siphamia* spp. eggs are average in size compared to the eggs of other apogonids and correspond with the general trend that fishes with larger broods have smaller eggs (Vagelli, 2011).

In a survey of sagittal otolith diameter as a percentage of fish  $L_S$  across 247 species in 147 marine fish families, Paxton (2000) determined that nearly half of the species with the largest otoliths ( $> 7\% L_S$ ) surveyed were luminous, including one species of apogonid

[*Archamia fucata* (Cantor 1849)], which had a larger otolith than its non-luminous counterpart examined [*Apogon aureus* (Lacépède 1802)]. This trend was true for most families with both luminous and non-luminous members (Paxton, 2000). The otolith diameter of *S. tubifer*, however, appears to be similar (*c.* 7%  $L_S$ ) to that of the non-luminous apogonid species examined by Paxton (2000).

The von Bertalanffy growth coefficientss for *S. tubifer* are similar to those reported for another relatively small cardinalfish, *O. rubrimacula* (Longenecker & Langston, 2006, Table III). Both fishes have similar asymptotic lengths ( $L_\infty$ ) and longevities < 1 year, but *S. tubifer* had an initial growth rate ( $K$ ) twice that of both *O. rubrimacula* and *O. doederleini* (Longenecker & Langston, 2006; Kingsford *et al.*, 2014). Additionally, the maximum age observed for *S. tubifer* in Okinawa was even shorter than those reported for two *Ostorhinchus* spp; the oldest observed *O. rubrimacula* in Fiji was 274 days (Longenecker & Langston, 2006), and the oldest *O. doederleini* reported in the southern Great Barrier Reef, Australia, was 368 days. Yet, much like this study, few fishes examined were older than 200 days (Kingsford *et al.*, 2014).

No indication of the timing of settlement was evident in the otolith microstructure of *S. tubifer*; however, the youngest individual observed was 31 days old and was similar in  $L_S$  (11.5 mm) to the settlement sizes of other apogonid species (Leis *et al.*, 2015). Assuming that *S. tubifer* settle directly onto the reef and immediately take up residence among the spines of a host urchin, the PLD of *S. tubifer* could be *c.* 30 days. This result is similar to that reported for the weed cardinalfish *Foa brachygramma* (Jenkins 1903) in Okinawa with a mean PLD of

30.6 days at 11 mm  $L_S$  (Ishihara & Tachihara, 2011), and it is relatively long in comparison to the PLDs reported for other species (Leis *et al.*, 2015). Some apogonids, however, undergo a two-phase recruitment process, settling first on to sand rubble habitat before eventually taking up residence on a continuous reef with adults (Finn & Kingsford, 1996). Thus far, there is no evidence to suggest that *S. tubifer* settles out of the plankton on to non-urchin habitat prior to taking up residence with adults at an urchin. There is also an undefined period of time (estimated as a few days) after *S. tubifer* embryos have hatched in the male's mouth, but prior to their release into the plankton as larvae (Dunlap *et al.*, 2009, 2012), which could have an influence on the PLD and should be considered in future studies.

There are few studies of the life histories and ecology of other bacterially luminous fishes. Previous studies either described aspects of the life history with no examination of the fish's symbiosis with luminous bacteria (Murty, 1986; Okuda *et al.*, 2005), or they focused primarily on the bioluminescent symbiosis (Hastings & Mitchell, 1971; Haygood, 1993); few studies have examined the growth of the light organ and the symbiont population relative to the growth of the host fish (Dunlap 1984; McFall-Ngai & Dunlap, 1984). This study shows that light organs of *S. tubifer* continue to increase linearly in diameter with *S. tubifer*  $L_S$ , and that the number of luminous symbionts housed within a light organ also increases throughout the life span of *S. tubifer* (Fig. 7). The maximum estimated symbiont population size in *S. tubifer*, however, was lower than that reported for leiognathids, monocentrids and anomalopids, and may be consistent with the generally smaller light organ of adult *S. tubifer* compared to light organs of adults of these other fishes (Haygood, 1993).

The symbiosis with *P. mandapamensis* does not begin immediately upon hatching in *S. tubifer* (Dunlap *et al.*, 2012); the light organ of *S. tubifer* becomes receptive to colonization by the symbiotic bacteria after 1 week or more of development post-release from the male's mouth (Dunlap *et al.*, 2012), and larvae that were 2.8 mm in  $L_S$  had no luminous symbionts in light organs, whereas larvae that were 3.5 and 10.4 mm  $L_S$  had symbionts (Leis & Bullock, 1986). It remains unknown how many bacterial cells initially colonize a light organ and for how long initial colonization is possible, but evidence from this study suggests that, once established, the population size of *P. mandapamensis* within a light organ increases throughout a host *S. tubifer*'s lifespan.

Before the current analysis of *S. tubifer* in Okinawa, *O. rubrimacula* in Fiji (Longenecker & Langston, 2006) was the smallest and most short-lived cardinalfish reported. Results of this study, however, suggest that *S. tubifer*, despite its similar size, is even more short-lived than *O. rubrimacula*, and once settled on a reef, grows at a rate twice that of both *O. doederleini* and *O. rubrimacula* (Longenecker & Langston, 2006; Kingsford *et al.*, 2014; Table III). Despite having similar lengths of asymptotic growth, *S. tubifer* reaches sexual maturity sooner than *O. rubrimacula* and reproduces over more of its lifespan, as reflected by the lower reproductive load in *S. tubifer* (Table III); however, the mean brood size of *S. tubifer* is much lower than that of *O. rubrimacula* (Longenecker & Langston, 2006). The rapid growth to maturity and short lifespan of *S. tubifer* correspond with its cryptic behaviour and support the hypothesis that high mortality, possibly due to predation, has influenced the ecology of this small, bioluminescent coral reef fish.

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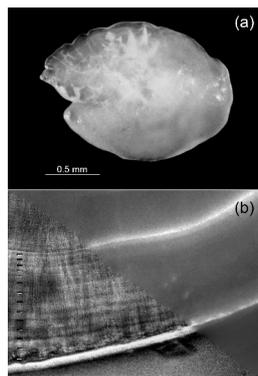


fig1

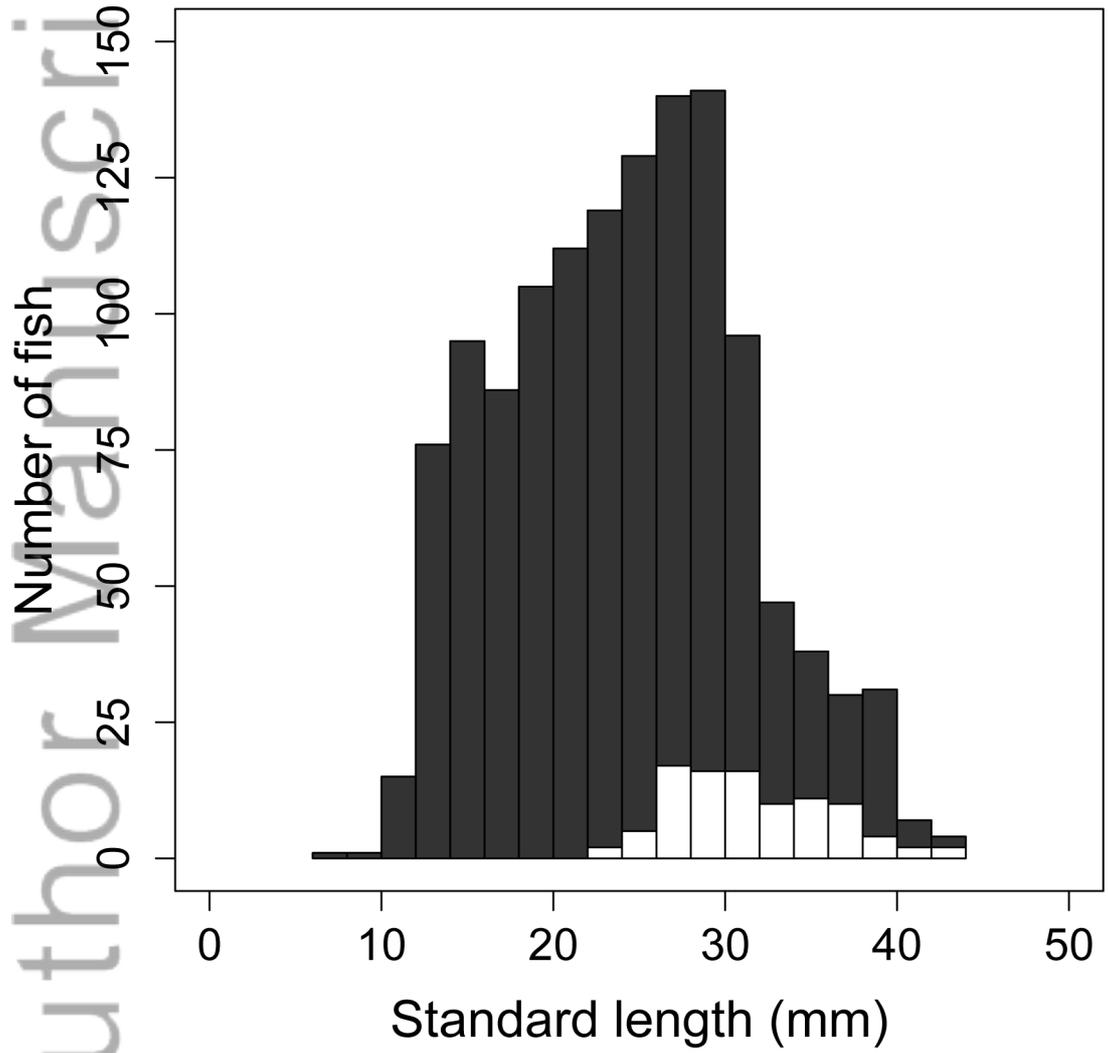


fig2

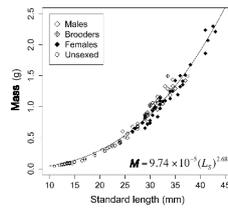


fig3

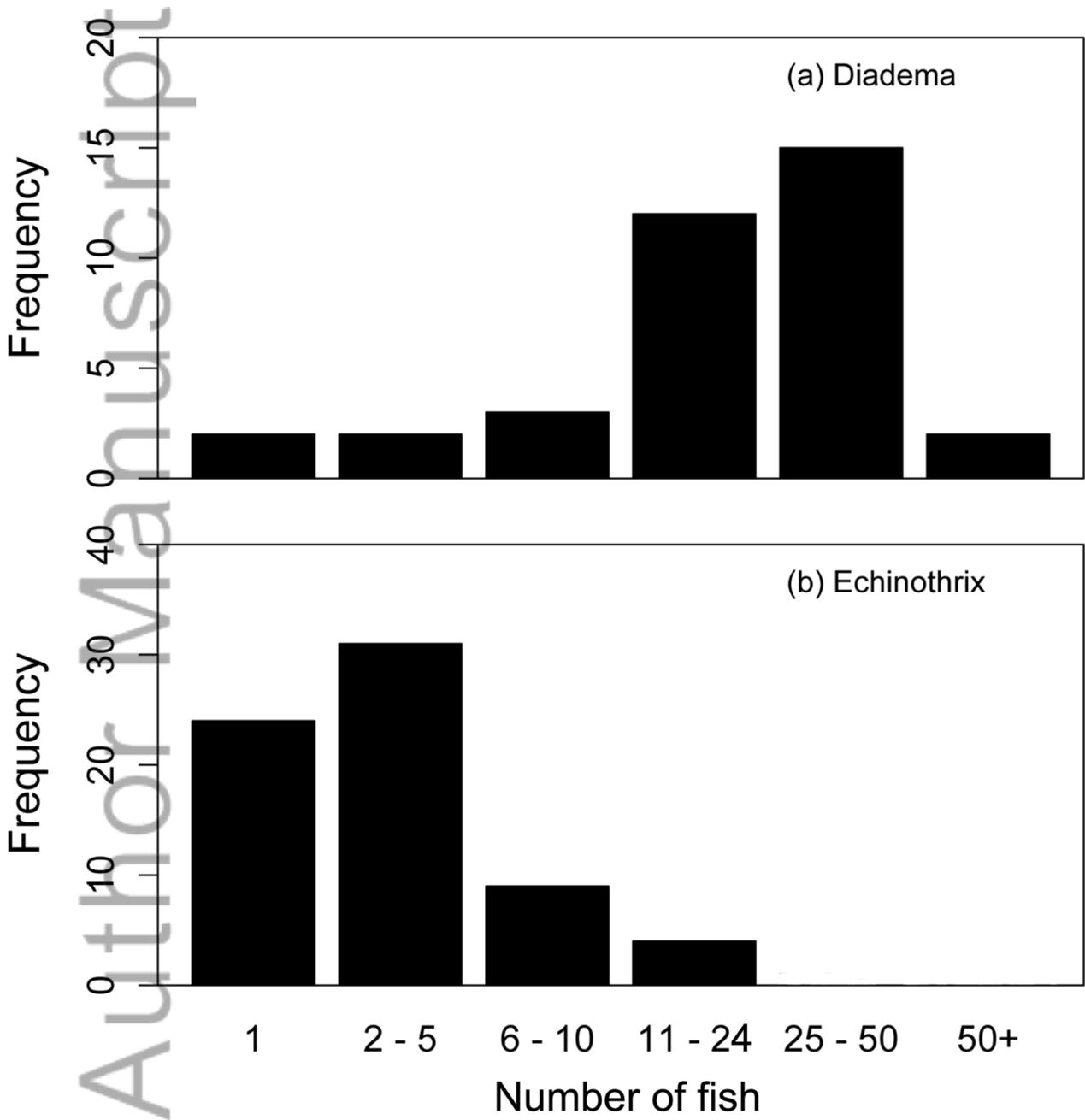


fig4

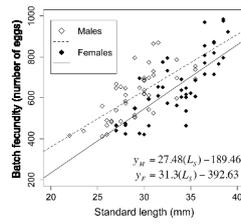


fig5

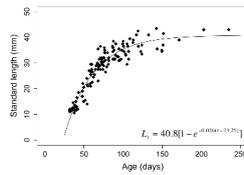


fig6

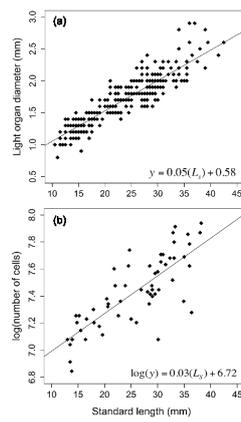


fig7

## Figure captions

FIG. 1. (a) Photograph of a right sagittal otolith of *Siphamia tubifer* (27 mm standard length,  $L_S$ ) and (b) overlay of two images of the outer edge of a sagittal otolith cross-section from an individual taken 15 days post-immersion in a tetracycline-seawater solution. For (b), the image at the left was captured under transmitted light and the image at the right was captured under ultraviolet light. The black tick marks indicate daily growth bands.

FIG. 2. Frequency histogram of standard length ( $L_S$ ) of *Siphamia tubifer* from various locations in Okinawa, Japan, from 2011 to 2014. open ,  $L_S$  frequencies of brooding males. [type-setter: please change 'fish' in Y axis label to '*S. tubifer*'. X-axis to  $L_S$  (mm)]

FIG. 3. Total wet mass ( $M$ ) of *Siphamia tubifer* as a function of standard length ( $L_S$ ;  $n = 121$ ) fitted with the curvilinear function  $y=9.74 \times 10^{-5}x^{2.68}$ . Open diamonds, males; crossed diamonds, brooders; closed diamonds, females; open circles, unsexed. [type-setter: please remove key and equation from the figure as I have added these to the legend.] X-axis to  $L_S$  (mm)

FIG. 4. The number of *Siphamia tubifer* aggregated together among the spines of a host sea urchin: (a) *Diadema setosum* and (b) *Echinothrix calamaris*. [type-setter: please remove 'Diadema' and 'Echinothrix' from a and b labels on figure. Please change 'fish' to '*S. tubifer*' on X axis label. Centre frequency between (a) and (b)]

FIG. 5. Batch fecundity of *Siphamia tubifer* represented as the number of fertilized eggs in the mouths of brooding males (open diamond dashed line;  $n = 46$ ,  $r^2 = 0.46$ ,  $F_{1,44} = 39.47$ ,  $P < 0.001$ ;  $y=27.48x-189.46$ ) and eggs in the ovaries of females (closed diamond solid line;  $n = 49$ ,  $r^2 = 0.63$ ,  $F_{1,47} = 82.59$ ,  $P < 0.001$ ;  $y=31.3x-392.63$ ) as a function of *S. tubifer* standard length ( $L_S$ ). [type setter: please remove key and equations from the figure as I have added these to the figure legend] X-axis to  $L_S$  (mm)

FIG. 6. Standard length ( $L_S$ ) of *Siphamia tubifer* by age as determined from counts of daily growth bands in sagittal otoliths fitted to the von Bertalanffy growth function  $y=40.8[1-e^{-0.026(x-23.25)}]$ . [type-setter: please remove equation from the figure as I have added it to the figure legend] y-axis to  $L_S$  (mm)

FIG. 7. (a) The diameter of a *Siphamia tubifer* light organ ( $n = 299$ ,  $r^2 = 0.82$ ,  $F_{1,297} = 1345$ ,  $P < 0.001$ ;  $y=0.05x+0.58$ ) and (b)  $\log_{10}$  number of luminous bacteria present in a light organ [ $n = 58$ ,  $r^2 = 0.62$ ,  $F_{1,56} = 95.25$ ,  $P < 0.001$ ;  $y=0.03x+6.72$ ] as a function of *S. tubifer* standard length ( $L_S$ ).

[type-setter: please remove equations from the figures as I have added them to the figure legend.]

X-axis to  $L_S$  (mm) log to  $\log_{10}$

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**Tables**

TABLE I. Summary of the diet of *Siphamia tubifer* ( $n = 25$ ) in Okinawa, Japan. Percentage gut content (mean  $\pm$  S.E.) is the mean percentage composition of each prey item of the total diet across all individuals of the standard length ( $L_S$ ) class indicated. Percentage occurrence (mean  $\pm$  S.E.) is the percentage of individuals in each size class in which that prey category was present in the diet. Rank indicates the relative importance of each prey item to the diet of each size class as a reflection of percentage occurrence combined with percentage content

Prey type	<i>S. tubifer</i> < 22 mm $L_S$ ( $n = 14$ )			<i>S. tubifer</i> > 22 mm $L_S$ ( $n = 11$ )		Rank
	Per cent gut content	Per cent occurrence	Rank	Per cent gut content ( $\pm$ S.E.)	Per cent occurrence	
Amphipoda	18.1 $\pm$ 4.4	64.3	2	10.1 ( $\pm$ 9.1)	18.2	6
Decapod crab megalops	2.8 ( $\pm$ 2.1)	14.3	8	6.0 ( $\pm$ 2.7)	36.4	4
Decapod crab zooea	8.9 ( $\pm$ 2.9)	50.0	3	5.1 ( $\pm$ 3.6)	18.2	8
Decapod shrimp	34.4 ( $\pm$ 8.0)	78.6	1	31.7 ( $\pm$ 9.0)	63.6	1

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Chaetognatha	0.7 ( $\pm$ 0.7)	7.1	10	-	-	-
Copepoda	11.3 ( $\pm$ 5.6)	35.7	5	1.5 ( $\pm$ 1.0)	18.2	9
Isopoda	-	-	-	0.6 ( $\pm$ 0.6)	9.1	13
Mollusca	-	-	-	0.7 ( $\pm$ 0.7)	9.1	10
Mysidacea	6.5 ( $\pm$ 5.3)	21.4	6	9.4 ( $\pm$ 3.6)	45.5	3
Ostracoda	0.5 ( $\pm$ 0.5)	7.1	11	0.7 ( $\pm$ 0.7)	9.1	10
Polychaeta	2.2 ( $\pm$ 1.2)	21.4	7	10.6 ( $\pm$ 5.6)	27.3	5
Stomatopoda	12.5 ( $\pm$ 7.2)	35.7	4	3.9 ( $\pm$ 3.1)	18.2	7
Tanaidacea	2.3 ( $\pm$ 1.6)	14.3	9	0.7 ( $\pm$ 0.7)	9.1	10
Teleost larvae	-	-	-	19.2 ( $\pm$ 9.5)	45.5	2
Fish eggs	-	-	-	0.6 ( $\pm$ 0.6)	9.1	13

TABLE II. Otolith growth band validation of *Siphamia tubifer*. Treatment refers to the number of days after tetracycline immersion that otoliths were sampled,

Treatment (days)	$n_i$	$L_S$ range (mm)	$n_f$	Mean $\pm$ S.E. count
5	12	27.5 - 41.0	1	5
10	15	27.5 - 41.5	5	10.8 $\pm$ 1.7
15	15	29.0 - 37.5	5	14.6 $\pm$ 2.7

$L_S$ , standard length;  $n_i$  and  $n_f$ , the numbers of *S. tubifer* treated and the number of otoliths recovered with visible UV bands.

TABLE III. The von Bertalanffy growth coefficients,  $L$ ,  $K$  and  $t_0$ , available for apogonids. Minimum size and time at maturity,  $L_m$  and  $t_m$ , and maximum size and longevity,  $L_{max}$  and  $t_{max}$  are also listed. Reproductive load,  $L_m:L$ , was calculated when possible. Lengths in bold are reported as total lengths,  $L_T$ , and times or rates in bold are in years; all other lengths are reported as standard lengths,  $L_S$ , and times or rates are in days. Where two values are present, data are males (females)

Species	$L$ (mm)	$K$ (time <sup>-1</sup> )	$t_0$	$L_m$ (mm)	$t_m$	$L_{max}$ (mm)	$t_{max}$	$L_m:L$	Location	Reference
<i>Apogon fasciatus</i>	<b>105.5</b>	<b>1.88</b>	<b>-0.04</b>	<b>46.4</b>	-	-	-	-	South-west Taiwan	Wu (2009)
<i>Apogon imberbis</i>	<b>120.5</b>	<b>0.41</b>	<b>-0.57</b>	<b>55</b>	<b>1</b>	<b>121</b>	<b>5</b>	0.46	North-west Mediterranean Sea	Raventós (2007)
<i>Apogon lineatus</i>	<b>86.6 (118.5)</b>	<b>1.12 (0.37)</b>	<b>-0.01 (-1.03)</b>	<b>53 (65)</b>	<b>1</b>	<b>103 (112)</b>	<b>3</b>	0.61 (0.55)	Tokyo Bay, Japan	Kume <i>et al.</i> (1998)
	<b>94.7 (85.0)</b>	<b>0.50 (1.23)</b>	<b>-0.88 (-0.07)</b>	<b>51.3 (65)</b>	<b>1</b>	<b>110</b>	<b>4 (5)</b>	0.54 (0.77)	Niigata Prefecture, Japan	Kume <i>et al.</i> (2003)
<i>Ostorhinchus doederleini</i>	65.04	0.01	-	-	-	74	368	-	Great Barrier Reef, Australia	Kingsford <i>et al.</i> (2014)
	86.5 (88.6)	<b>1.56 (1.62)</b>	<b>-0.02 (-0.02)</b>	69 (73)	<b>1</b>	92.5 *	<b>6 (7)</b>	0.80 (0.82)	Shikoku Island, Japan	Okuda <i>et al.</i> (1998)
<i>Ostorhinchus rubrimacula</i>	40.8	0.014	22.45 d	35	16 2	43 **	274	0.86	Koro, Fiji	Longenecker & Langston (2006)
<i>Pterapogon kauderni</i>	71	<b>0.74</b>	<b>-0.11</b>	40	<b>1</b>	66	<b>3-5</b>	0.56	Banggai Islands, Indonesia	Ndobe <i>et al.</i> (2013)
<i>Siphamia tubifer</i>	40.8	0.026	23.25	22	53	43.5	240	0.54	Okinawa, Japan	this study

\*largest size + S.D. reported

\*\*approximate size estimated from growth data

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