Pelagic larval duration and population connectivity in New Zealand triplefin fishes (Tripterygiidae

Yair Kohn

Environmental Biology of Fishes

Environ Biol Fish (2011) 91:275–286
DOI 10.1007/s10641-011-9777-3

Pelagic larval duration and population connectivity in New Zealand triplefin fishes (Tripterygiidae)

Yair Y. Kohn · Kendall D. Clements

Received: 4 July 2010 / Accepted: 1 December 2010 / Published online: 5 March 2011

Abstract The relationship between pelagic larval duration (PLD) and population connectivity in marine fishes has been controversial, but most studies to date have focused on tropical taxa. Here, we examine PLD in 11 species of triplefin fishes from a temperate environment in the Hauraki Gulf, New Zealand, to describe daily increment patterns and settlement marks in the otoliths. The formation of daily increments was validated using larvae of known age and tetracycline marking of settled juveniles. Settlement mark identity was verified by comparing total increment counts from otoliths of recently settled fishes with PLD counts from post-settlement fishes. A similar pattern of three groups of increments across the otolith was found in all specimens examined. The settlement mark was similar in all species and occurred as a sharp drop in increment width within the area of transition in optical density. PLD was lengthy, compared to species of triplefins from elsewhere, and ranged between 54.4±1.7 SE days in *Bellapiscis lesleyae* to 86.4±2.6 SE days in *Forsterygion malcolmi*. Variation in PLD within species was high but did not mask interspecific differences. PLD was not phylogenetically constrained, as sister species differed significantly in PLD. PLD was compared with genetic population connectivity for eight of the study species using mitochondrial gene flow data from Hickey, Lavery, Hannan, Baker, Clements. Mol Ecol 18:680–696 (2009). The observed lack of correlation between PLD and gene flow suggests that dispersal is limited by other factors, such as larval behaviour and the availability of settlement habitat.

Keywords Pelagic larval duration · Population connectivity · Otolith microstructure · Triplefin fishes

Y. Y. Kohn (✉) · K. D. Clements
School of Biological Sciences, University of Auckland,
Private Bag,
92019 Auckland, New Zealand
e-mail: kohya760@student.otago.ac.nz

Present Address:
Y. Y. Kohn
Zoology Department, The University of Otago,
340 Great King St.,
9016 Otago, New Zealand

Introduction

The relationship between pelagic larval duration (PLD) and population connectivity in marine fishes has been controversial (Macpherson and Raventos 2006). PLD was, until recently, considered a "black box" and there was little understanding of the processes that influence larval dispersal and settlement (Cowen and Sponaugle 2009). Traditionally, larvae were thought of as passive particles that drifted with the currents like plankton (Fuiman 2002). Recent work on reef fishes has shown that both physical processes and biological traits affect dispersal, and

that understanding the complex interaction between these is necessary to explain population connectivity (Cowen and Sponaugle 2009; Giovannotti et al. 2009). However, most of the work on PLD and connectivity in fishes has concentrated on a relatively small number of taxa that either inhabit low latitudes and/or are characterized by low dispersal potential (Bradbury et al. 2008), notably pomacentrids and labrids (e.g., Victor 1986a; Wellington and Victor 1989; Victor and Wellington 2000; Bay et al. 2006).

Recent studies have shown that fish larvae develop both swimming and sensory abilities during the pelagic phase. Larvae of both tropical (Fisher 2005) and warm-temperate marine and estuarine fishes (Clark et al. 2005) can actively influence their dispersal by swimming at speeds greater than the mean speed of currents encountered in their oceanic environment (Leis and Carson-Ewart 1997; Leis et al. 2006; Leis et al. 2009). The sensory systems of larval fishes are highly developed, particularly in the late stages of larval growth (Fuiman 2002). Fish larvae are responsive to a variety of sensory cues including reef sounds (Tolimieri et al. 2000), light (Job and Bellwood 2000), chemical cues (Atema et al. 2002; Døving et al. 2006; Gerlach et al. 2007) and floating objects (such as seaweed) (Kingsford 1992). Larvae that are developed enough to settle are considered to be in their "competent stage" (Victor 1986b), and variation in settlement times (i.e. PLDs) may occur by prolonging this competent stage. Larvae can delay settlement in response to restricted availability of suitable settlement habitat (Jenkins and May 1994), or when held in pelagic cages and thus prevented from reaching the substratum (McCormick 1999).

PLD can influence gene flow between populations because of its potential to affect dispersal (Doherty et al. 1995; Riginos and Victor 2001; Purcell et al. 2006; Bradbury et al. 2008). However, this influence is often masked by other factors such as oceanic features (Galarza et al. 2009), habitat compatibility (Rocha et al. 2005), larval retention (Bowen et al. 2006; Choat 2006; Almany et al. 2007) and phylogenetic constraints (Bay et al. 2006). Furthermore, Helfman et al. (2009) have pointed out that most of the comparisons on the relationships between PLD and population structure are confounded by the inclusion of phylogenetically distinct reef fish lineages.

Triplefins (Tripterygiidae) are a family of 163 species from 29 genera (Patzner et al. 2009) that are found worldwide in tropical, temperate to polar regions (Nelson 2006). In New Zealand, 26 species of triplefins have been described from 12 genera (Patzner et al. 2009). All species are endemic, although three of these have been introduced to Australian waters (Hickey et al. 2004). Most of these species are closely related, and the New Zealand triplefins have diversified considerably in habitat use (Feary and Clements 2006; Wellenreuther et al. 2007, 2008), physiology (Hickey and Clements 2003; Hilton et al. 2008), and to a lesser extent in diet and trophic morphology (Feary et al. 2009).

Triplefin larvae are a numerically dominant component of coastal larval fish assemblages in New Zealand, and as a result a number of studies have examined their horizontal and vertical distribution, demonstrating that the larvae of some species occur well offshore (Kingsford and Choat 1989; Kingsford 1992; Tricklebank et al. 1992; Hickford and Schiel 2003). PLD in these studies was not estimated directly, but was inferred to be between 2 and 3 months. Recently, Shima and Swearer (2009) used otolith microchemistry to demonstrate a high degree of local retention of larvae in a population of *Forsterygion lapillum*.

Hickey et al. (2009) demonstrated considerable interspecific variation in levels of mitochondrial gene flow (Φ_{ST}) among eight species of New Zealand triplefins. Three species displayed little phylogeographic pattern, three species fitted an isolation-by-distance model, and the remaining two species exhibited strong phylogeographic structuring. These differences in gene flow suggest that there is considerable interspecific variation in larval dispersal in New Zealand triplefin species, yet PLD is known for only one of these species, i.e. *F. lapillum* (Shima and Swearer 2009). Collectively, these studies indicate the complexity of factors governing dispersal in New Zealand triplefins, at least some of which have the potential to disperse over long distances as larvae despite the highly philopatric, demersal lifestyle of the adults (Thompson 1979; Subedar 2009). Here, we aim to evaluate the importance of PLD with regard to connectivity in this group of temperate fishes by estimating PLD in 11 species, and examining these data in the context of previous work on larval behaviour and gene flow.

Materials and methods

Study species and sampling

Triplefin specimens were collected from the Hauraki Gulf, New Zealand (175°25′E, 36°10′S) mostly between October and January of 2005 and 2006, with the exception of 12 individuals collected between 1998 and 2003. This sampling period was chosen as it was the estimated settlement time for most species considering that peak spawning in most species is around August (Wellenreuther and Clements 2007). In total, 11 species of New Zealand triplefins were examined for PLD. Of these, eight species were used to examine the relationships between PLD and on population connectivity using data available from Hickey et al. (2009) (see below).

The collection method and location depended on the species concerned. (1) Specimens of *Bellapiscis* (*B. lesleyae* and *B. medius*) were collected from intertidal rock pools using hand nets. (2) Some specimens of *Forsterygion* (*F. capito*, *F. gymnota* and *F. nigripenne*) were collected using bait catchers (manufacturer: Sea Harvester Tackle) baited with crushed mussels. The bait catcher was placed in depths of 0.5–3 m and retrieved after 15 min. (3) Subtidal reef species (*F. lapillum, F. malcolmi, F. maryannae, F. varium, Notoclinops segmentatus* and *Ruanoho whero*) were collected using SCUBA gear and hand nets/slurp guns in depths of 2–20 m. (4) *Forsterygion lapillum* were also caught using 'Standard Monitoring Units for the Recruitment of Reef Fishes' (SMURFs) (Ammann 2004). These devices were anchored about half a meter from the bottom to a meter away from kelp forest in two separate locations. The SMURFs were left for a period of 2–7 days between collections. Upon removal from the water, all specimens to be used in the study were placed in a seawater and clove oil solution. Samples were subsequently preserved in 70% ethanol.

Otolith preparation and analysis

In total, 147 otoliths from 11 species of New Zealand triplefins were used for analysis. Sample size was between 8 and 17 otoliths per species (Table 1). Fish were weighed and measured for standard length (SL) and total length (TL). Sagittal otoliths were removed under a dissecting microscope (Nikon™ 5MZ-2T), then cleaned with ethanol and stored dry (Hernaman et al. 2000). Sagittae were then weighed and ground (see below). Only one of the two sagittae was ground unless the first sagitta did not give clear results (Wilson and McCormick 1997). Lapilli (n=20) from six species were also removed and examined to determine whether daily increments were clearer in sagittae or lapilli (Fowler 1989).

Only sagittae were used for further analysis using the method of Wilson and McCormick (1997). Sagittae were mounted on glass slides using preheated CRYSTAL BOND™ 509 Clear Mounting cement in such a way that the distal end protruded over the edge of the slide. Mounted sagittae were then ground using a either a Gemmasta™ faceting machine or sand paper (800 and 1,200 grit) at a right angle until the nucleus was exposed. Sagittae were then removed and remounted face down with the polished face facing the slide and ground to achieve a thin transverse section. Sections were then polished using lapping films (12 μm and 3 μm).

Daily increments were counted and measured for width by using an image analysis system consisting of a compound microscope (LEICA™ DMR, magnification 1,000×, otoliths were imbedded in immersion oil) with a digital camera (LEICA™ DC 500) attached to it and image analysis software (analySIS®). Otolith increments were counted along the long axis of the otolith from the nucleus to the edge. In many cases not all increments were visible along the same axis, and therefore counts were made following the closest possible trajectory (Campana 1992).

Validation of settlement marks and daily increments

Validation of settlement marks is required for two main reasons. First, settlement marks may be absent (or at least not visible) in the otoliths of some fish species. Settlement marks are identified mainly in fishes that undergo a dramatic change in environment during their settlement, i.e. from pelagic to benthic (Morales-Nin 2000). Second, several types of settlement marks have been reported in the literature (four types were described by Wilson and McCormick (1999)). The differences between these involve different increment patterns (regarding width and optical density). Otoliths may have irregular patterns of increments for other reasons (e.g. temperature,

Table 1 PLD of the 11 triplefin species examined in the present study

Species (n)	Mean PLD±SE (days)	Min PLD (days)	Max PLD (days)
B. lesleyae (16)	55.68±1.68	41	67
N. segmentatus (16)	56.5±0.91	49	64
F. maryannae (16)	58.37±1.41	48	69
R. whero (14)	63.92±1.71	54	75
F. lapillum (17)	64.94±2.38	51	84
F. varium (13)	65±2.32	53	79
F. gymnota (8)	68.5±1.56	62	77
F. nigripenne (12)	69.41±2.28	59	83
F. capito (9)	74.33±2.48	67	89
B. medius (16)	76.87±3.21	61	114
F. malcolmi (10)	86.4±2.55	78	100

feeding, and other life history events). A validation confirms the identification of the settlement mark.

Recently settled triplefins were identified by their small size (<30 mm, McDermott and Shima 2006) and relative lack of pigmentation (Connell and Jones 1991). Fishes between 30 mm and 50 mm TL were considered juveniles. Recently settled fish were chosen a priori for settlement mark validation tests. The total increment count of recently settled fishes was compared with the PLD of older juvenile conspecifics (Raventós and Macpherson 2001). Recently settled specimens from five species were suitable to validate settlement marks: *Bellapiscis medius* (n=3), *Forsterygion lapillum* (n=4), *F. malcolmi* (n=2), *F. maryannae* (n=5) and *Notoclinops segmentatus* (n=5).

Daily increments and settlement marks have not previously been confirmed in New Zealand triplefins, and it was thus necessary to demonstrate that the increments identified as daily were formed in a 24 h cycle (Geffen 1992). The reason for validation is not so much due to doubt that daily increments are formed (Brothers et al. 1983), but rather the possibility of confusing them with check marks and sub-daily increments.

Tetracycline

Initially, fish were immersed in tetracycline hydrochloride 95% (SIGMA™ ALDRICH) (Walker and McCormick 2004), but this method did not yield results. Subsequently, fish were injected with tetracycline as follows. Captured fishes were placed in seawater and transported to the seawater facility in the School of Biological Sciences, University of Auckland. Fish were placed in 60 L aquaria connected to a recirculating sea water system (temperature 15°C, salinity between 34‰ and 36‰). Fish were acclimated for a period of at least 24 h and were fed daily using a mixture of brine shrimp, pink shrimp, krill and mysis shrimp (Biosuppplies, Auckland). Injection was conducted on two groups. Six individuals from three species (*F. lapillum*, n=2, *Forsterygion varium*, n=2, *Ruanoho whero*, n=2) were used for the first experiment. Nine individuals of the species *F. capito* were used for the second. A solution of tetracycline hydrochloride and saline (5 mg tetracycline per 1 ml saline) was prepared. Fish were anaesthetized using clove oil (Griffiths 2000) and weighed. The solution was then injected into the peritoneal cavity at a concentration of 50 mg tetracycline per 1 kg of fish (Hernaman et al. 2000). Fish were then returned to the aquarium, which was covered to prevent entry of light (Walker and McCormick 2004). After 24 h, the cover was removed and fish were returned to a 16D: 8L cycle. Fish were then kept for an additional 13 or 14 days and then killed using clove oil (see above) (Griffiths 2000). Otoliths were removed, wrapped in aluminium foil and stored dry (Hernaman et al. 2000). Otoliths were examined under a compound microscope using UV light.

Larval otoliths

Eggs were collected from a *Forsterygion nigripenne* nest in another aquarium (15°C, salinity between 34‰ and 36‰, 16D:8L cycle) and were placed in a floating cylinder in a separate tank with the same

rearing conditions. Larvae hatched between 1 and 10 days after removal. After hatching, larvae were observed every 24 h and dead larvae were removed and labelled. Otoliths (n=3) were removed by using fine syringe needles, and placed on a slide and were embedded in immersion oil. Sagittae were used for analysis as described above except that there was no need to grind larval otoliths. Daily increments were clearly visible under the microscope (magnification 1000×). Despite the relatively small sample size here (e.g. cf Aldanondo et al. 2008), we feel that this additional validation was useful as it demonstrates daily increment formation in this species, and because it enabled us to identify the hatch mark formed on the first day after hatching.

Data analysis

Statistica (StatSoft®) was used for statistical analysis. One-way ANOVA and post-hoc Tukey tests were performed to detect differences in PLD between the 11 species examined. A repeated measures ANOVA was performed to test the hypothesis that the increments observed in the transition area (identified by a change in optical density) also displayed a change in increment width pattern (Wilson and McCormick 1999). Each consecutive increment was treated as a repeated measurement. The null hypothesis was therefore that all increments in the transition area were of the same width (i.e. variation was statistically insignificant). The logic behind this test is that unless the transition area marks a dramatic event in the fish's life there is no reason to assume that differences in increment width will reoccur at the transition area (Wilson and McCormick 1999). A post-hoc Tukey test was performed to test the differences between increments.

For validation of settlement marks, a paired t-test was used to compare the PLD of individuals with apparent settlement marks on their otoliths with the total increment count of otoliths from recently or pre-settled conspecifics.

Results

Pattern of daily increments

All the sagittae (n=147) that were analyzed displayed a similar pattern of daily increment formation. In general, the pattern of increments across each otolith was divided into three major groups according to increment width, optical density and position relative to the nucleus. (1) Increments close to the nucleus were very fine, narrow (between 0.5 and 2 μm) and dark. (2) Increments gradually became wider and peaked at 5–10 μ and were dark. (3) Increments decreased in width and reached 2–4 μm, and were lighter than the previous increments (Fig. 1).

A transition in optical density of increments was apparent between groups two and three, where earlier increments appeared darker under the compound microscope than later increments (Fig. 1). This transition corresponded to the decrease in increment width.

Sub-daily increments were found in otoliths of all species examined. Sub-daily increments disappeared when there was a change of focus, while daily increments remained clear (cf. Victor 1986a). Furthermore, sub-daily increments were often observed only on some portion of the otolith (cf. Victor 1986a). Therefore, it was always possible to compare sub-daily increments with adjacent daily increments.

Validation of daily increments

Larval otoliths

Three *Forsterygion nigripenne* larvae that hatched on 15/9/06 and died after 5 days were dissected to recover sagittae. All sagittae had four clear increments plus a fifth partial increment (Fig. 2), validating daily increments. In addition, the number of increments indicates that the first increment (the hatch mark) was laid down on the first day post-hatch.

Tetracycline

All fishes (n=15) survived the tetracycline treatment over the 13 or 14-day post-injection period except one fish (*Ruanoho whero*) that died after 6 days. A fluorescent mark was clearly visible under UV light in the sagittae of all fishes injected with tetracycline.

Daily increments following the fluorescent mark were evident in all sagittae examined. However, it was difficult to distinguish between increments in all of these otoliths. Increments on four of the 15 otoliths examined were clear enough to read. There was a good agreement between the expected (i.e. 13 or 14)

Fig. 1 *Forsterygion nigripenne* otolith microstructure. Transverse section of sagitta showing the three groups of increments: (i) increments close to the nucleus are fine, narrow (0.5–2 μm), and dark (*right*); (ii) intermediate increments are wider (5–10 μm) and dark (*middle*); and (iii) the transition area is characterized by narrower (2–4 μm), lighter increments (*left*, the settlement mark is indicated by the *arrow*)

and observed number of daily increments after the tetracycline mark (Table 2, chi-square = 0.384, p=0.057).

Settlement marks

A settlement mark was identified as a change in increment width from wide to narrow within the area of transition in optical density (i.e. from dark to clear). Measurements of the width of these increments showed an abrupt change in width in all 11 species examined (e.g. Fig. 3). The settlement marks observed in this study correspond to the type Ia settlement mark described by Wilson and McCormick (1999). Daily increments before and after the settlement mark differed significantly in width in all species except *F. maryannae*, but nevertheless the change in optical density seen in all other species examined (i.e. the settlement mark) was observed in all otoliths of *F. maryannae* examined.

Validation of settlement marks

Otoliths of recently settled fishes displayed either no transition area (i.e. settlement mark) or a transition area that was at the very edge of the otolith (Fig. 4). Otoliths of juvenile fishes contained a clear settlement mark.

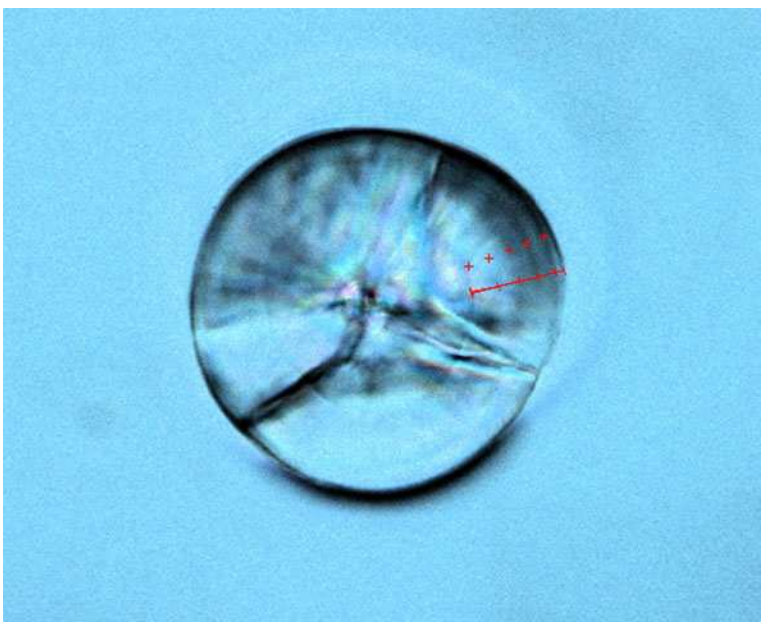

Fig. 2 *Forsterygion nigripenne* larval otolith. Sagitta of *F. nigripenne* larva that lived for 5 days. Four complete increments are visible plus a partial fifth increment forming on the edge of the otolith. The hatch mark is also visible (marked as the first *red* "+")

Table 2 Validation of daily increments. Validation of daily otolith increments in four specimens of three triplefin species injected with tetracycline. "Expected" represents the number of days fish were retained after injection. "Observed" is the number of increments observed after the tetracycline mark

Species (n)	Expected	Observed
Forsterygion capito (1)	13	14
F. capito (1)	13	15
F. varium (1)	14	14
Ruanoho whero (1)	6	6

The total increment count of recently settled fish was compared with the PLD of older juvenile conspecifics to validate the settlement mark in each species. In one of the five species examined there was a statistically significant difference between the two test groups (*Forsterygion lapillum* p=0.007), thus the settlement mark was not validated in this species. These *F. lapillum* were captured using SMURFs (see discussion). In the other four species there was no significant difference between the two test groups (*Bellapiscis medius* p=0.1455, *F. malcolmi* p= 0.1506, *F. maryannae* p=0.0894 and *Notoclinops segmentatus* p=0.438) thus validating the settlement marks. We note that the power of the test was low in three of the four validated species (*B. medius* = 35.9%, *F. malcolmi* = 14.3% and *F. maryannae* = 56.6%), and therefore results should be treated with caution. The power of the test for *N. segmentatus* was higher (83.3%).

PLD

PLD ranged between 41 (minimum PLD of *Bellapiscis lesleyae*) and 114 days (maximum PLD of *B. medius*) (Table 1). Mean species PLD ranged between 55.6 days in *B. lesleyae* to 86.4 days in *Forsterygion malcolmi* (Table 1).

PLD did not appear to be correlated with phylogenetic relationship. Some congenerics had statistically similar mean PLDs, e.g. *Forsterygion gymnota* 68.5 days and *F. capito* 74.3 days (p=0.948). The sister species *F. lapillum* and *F. nigripenne* had similar PLDs (64.9 days and 69.4 days, respectively, p=0.923). In contrast, the two other sister species pairs examined had significantly different mean PLDs, i.e. *Bellapiscis lesleyae* 55.6 days and *B. medius* 76.8 days, and *F. maryannae* 58.3 days and *F. malcolmi* 86.4 days (p<0.0001 in both cases). Mean PLD was strongly correlated with both maximum PLD (r=0.873, p=0.0004) and with minimum PLD (r=0.951, p<0.0001).

Discussion

The PLD (Table 1) of the 11 species of triplefins examined in this study is consistent with previous literature estimates of 2–3 months based on the interval between nesting and settlement (Thompson 1979; Kingsford and Choat 1989). Other species of triplefins examined from the Mediterranean (Raventós and Macpherson 2001), the Gulf of California (Riginos and Victor 2001) and Hawaii (Longenecker and Langston 2005) displayed a much shorter PLD of between 14 and 30 days. Indeed, the PLD of triplefin species examined in this study is long compared to most reef fishes in general. These results support recent findings demonstrating a negative correlation between temperature and PLD in various marine taxa including fishes (O'Connor et al. 2007; Bradbury et al. 2008).

Validations of daily increments and settlement marks

It is unlikely that the settlement mark in *Forsterygion lapillum* was misidentified, since it was similar to the

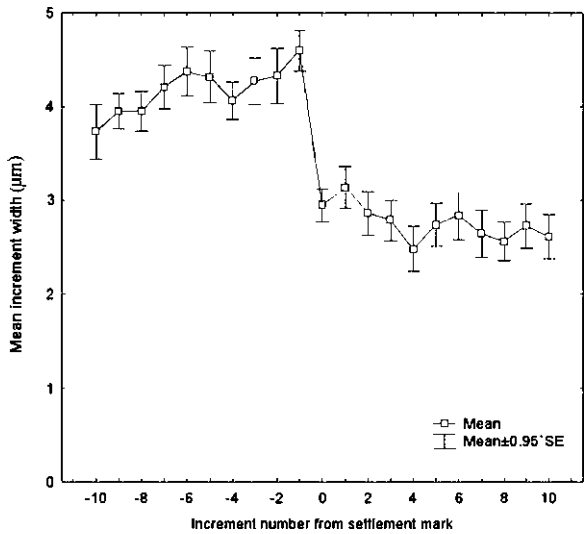

Fig. 3 *Bellapiscis lesleyae* settlement mark profile. Profile of mean increment width of ten increments from both sides of the settlement mark (n=16), which is represented by the decrease in width between increments −1 and 0

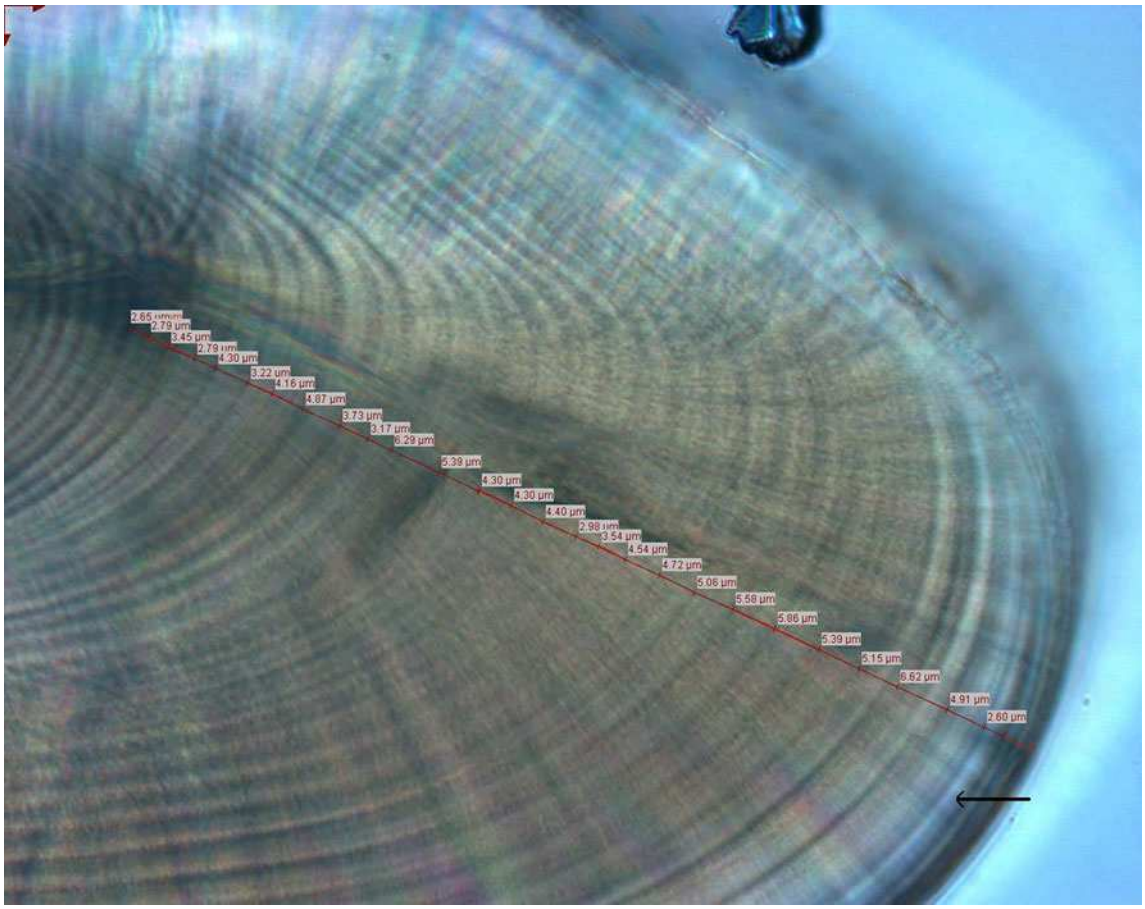

Fig. 4 *Bellapiscis medius* otolith of a recently settled fish. Transverse section of sagitta of a recently settled *B. medius*. Dark and wide increments are visible up to the edge of the otolith. The possible beginning of the transition zone is visible on the very edge of the otolith where the final visible increments are narrower and lighter (indicated by *arrow*)

settlement mark of all other species examined. Rather, there is a possibility that the specimens of *F. lapillum* that were used for total increment counts were caught at a stage earlier than that at which normal settlement onto reefs takes place. This is possible, since the *F. lapillum* specimens used for validation were the only triplefins in this study that were caught using SMURFs (Ammann 2004). This device was suspended in mid water, and therefore is more likely to capture pelagic larvae than the other collection methods used here. Although SMURFs mainly collect new recruits, pre-settlement larvae have also been collected using these devices (Vallès et al. 2008). The *F. lapillum* used for validation (i.e. from SMURFs) had very little pigmentation and were smaller (between 18.03 mm and 19.42 mm TL) than the size at settlement reported for this species (20 mm, Paulin and Roberts 1992). Interestingly, the mean PLD we found for SMURF-collected *F. lapillum* (52.2 days) is similar to that found in another study that used a similar method (51.3 days) (Shima and Swearer 2009).

A statistically significant difference in width was found between pre- and post-settlement increments on the otoliths of all species examined except those of *Forsterygion maryannae*. It is important to note that although increment width did not significantly vary across the transition area in this species, the change in optical density seen in all other species examined (i.e. the settlement mark) was observed on all otoliths of *F. maryannae* examined. Interestingly, *F. maryannae* is the only triplefin species that exhibits semi-pelagic behaviour (Feary et al. 2009). It is usually observed suspended in mid water close to the reef in schools of several to thousands of fish (Russell 1983; Hardy

Table 3 Study species: Data on π (genetic diversity) and Φ_{ST} from Hickey et al. (2009). Data on adult habitat depth, habitat and geographic range from Thompson (1979); Paulin and Roberts (1992); Fricke (1994); Syms (1995); Willis and Roberts (1996); Francis (2001); Hickey and Clements (2003); Feary and Clements (2006); Wellenreuther et al. (2007, 2008, 2009) and Hilton et al. (2008)

Species (n)	Mean PLD±SE (days)	π±SE	Φ_{ST}	Adult habitat depth (m)	Habitat	Geographic range
B. lesleyae (16)	55.68±1.68	0. 007±0.002	0.445	0–4	Lower intertidal pools and surge zone	Three Kings Is. to Stewart Is., Chatham Is.
N. segmentatus (16)	56.5±0.91	no data	no data	2–30	Rocky reefs	North Is. to Stewart Is.
F. maryannae (16)	58.37±1.41	no data	no data	2–30	Schools above rocky reefs	Three Kings Is. to Snares Is.
R. whero (14)	63.92±1.71	0.016±0.004	0	0–30	Cobbles and crevices on rocky reefs	Three Kings Is. to Snares Is. Chatham Is.
F. lapillum (17)	64.94±2.38	0.009±0.001	0.150	0–10	Rock or cobble on reefs and intertidal pools	North Is. South Is. and Stewart Island
F. varium (13)	65±2.32	0.019±0.001	0.156	0–30	Rocky reefs	Three Kings Is. to Snares Is., Chatham Is.
F. gymnota (8)	68.5±1.56	0.033±0.001	0	0–10	Reefs in turbid coastal locations	North Is. South Is., Antipodes Is., Chatham Is.
F. nigripenne (12)	69.41±2.28	0.010±0.000	0.834	0–3	Estuaries and river mouths	North Is., South Is. and Chatham Is.
F. capito (9)	74.33±2.48	0.015±0.001	0.31	0–12	Intertidal pools and upper subtidal	North Is., South Is., Stewart Is., Snares Is. Auckland Is., Antipodes Is., Chatham Is.
B. medius (16)	76.87±3.21	0.005±0.000	0.573	0–2	Intertidal pools	Three Kings to Stewart Is.
F. malcolmi (10)	86.4±2.55	no data	no data	5–35	Overhangs and crevices on rocky reefs	Three kings Is. to Stewart Is., Chatham Is.

1987; Francis 2001). Also, unlike other triplefins, this species feeds mainly on zooplankton (Feary et al. 2009). Furthermore, *F. maryannae* exhibits several paedomorphic characters, and thus resembles a larval *Forsterygion* in many respects (Hickey and Clements 2003).

PLD and population connectivity

Mean PLD was plotted against the Φ_{ST} scores of the eight species studied by Hickey et al. (2009) (Table 3). Φ_{ST} is a measure of gene flow that incorporates the distances between different haplotypes, i.e. nucleotide diversity (cf. F_{ST}, which is based on haplotype proportions, and in which haplotypes are considered equidistant). Φ_{ST} allows for haploid transmission of mitochondrial genomes (Excoffier et al. 1992).

PLD and population connectivity showed little correlation (r^2=0.0672). For example, *Forsterygion nigripenne* has a very high Φ_{ST} score of 0.834, indicating very little gene flow between populations, yet this species had a comparatively lengthy PLD of 69.4 days (Tables 1 and 3). PLD thus appears to be a poor predictor of population connectivity in New Zealand triplefins. It is possible that this result was influenced by the fact that our sampling was restricted to warmer, northern waters, and indeed it is likely that triplefins from higher latitude locations have a longer PLD due to the effect of colder temperatures on development (Bradbury et al. 2008). However, the species examined in this study share very similar geographic ranges (Table 3), being found throughout coastal New Zealand (Paulin and Roberts 1992; Fricke 1994). The inclusion of southern samples in the analysis would thus be unlikely to change the relationship between PLD and Φ_{ST}, as mean PLD would be expected to increase proportionately in all species.

It is likely that interspecific differences in larval behaviour and habitat availability obscure the potential influence of PLD on dispersal in triplefin species.

Carreras-Carbonell et al. (2006) showed that population connectivity in the Mediterranean triplefin *Tripterygion delaisi* depended on habitat availability. Such may also be the case in the sister species *Forsterygion nigripenne* and *F. lapillum* from the present study. The spatial scale of available habitat for *F. nigripenne* could explain the low level of population connectivity in this species. *F. nigripenne* is the only triplefin species found in estuaries (Wellenreuther et al. 2007), which are patchily distributed around coastal New Zealand (McLay 1976). Thus *F. nigripenne* is a specialist, restricted to habitats with low wave exposure and muddy substratum (Wellenreuther et al. 2007, 2009). In contrast, its sister species *F. lapillum* is a generalist species that is found in many types of habitats (Syms 1995; Feary and Clements 2006; Wellenreuther et al. 2007, 2008, 2009). *F. nigripenne* and *F. lapillum* have similar PLDs (69.4 and 64.9 days, respectively). However, *F. nigripenne* has a much higher Φ_{ST} score than *F. lapillum* (0.83 vs. 0.15, respectively). Thus, although larvae of these two species have the potential to disperse over similar distances, the interaction between habitat availability and larval behaviour may facilitate greater gene flow between populations of *F. lapillum* than between populations of *F. nigripenne*. An alternative explanation for the low levels of gene flow in *F. nigripenne* is that the larvae of this species return to their natal estuary after the pelagic phase. There is evidence of larval retention in triplefin species from other parts of the world (Brogan 1994 (California); Sabatés et al. 2003; López-Sanz et al. 2009 (Mediterranean)).

Hickey et al. (2009) found a positive correlation between the habitat depth of adults and Φ_{ST} in New Zealand triplefin species, suggesting that populations of species from shallow waters are more structured than those from deeper water. This strengthens the idea that habitat plays a role in gene flow in New Zealand triplefins. We found no correlation between PLD and adult habitat depth (r^2=0.0153, depth data taken from Wellenreuther et al. (2007)), although such a relationship has been demonstrated in other marine fishes (Bradbury et al. 2008).

Finally, the variation in dispersal distances may be greater in fishes with long PLDs, such as those found in the present study. The longer fish remain as larvae in the pelagic environment the more they are exposed to factors (e.g. currents, oceanographic barriers, temperature, food availability and predation) that could limit their dispersal and affect their survival (Pineda et al. 2007). Dispersal may therefore be expected to scale with the length of PLD only when it is very short (a few days). Furthermore, low Φ_{ST} scores can be caused by a relatively small number of individuals with extremely long PLDs, as genetic homogeneity between populations can be maintained by migration of only a few individuals per generation (Slatkin 1993). Further work on dispersal and connectivity is required to explain the apparent paradox of how a clade of fishes which share overlapping, sympatric distributions (Wellenreuther and Clements 2007) evolved such comparatively high diversity (i.e. 26 species) in a restricted geographic setting.

Acknowledgements We thank B. Doak for skippering the RV 'Hawere', Z. Hilton, S. van Dijken, L. Clements and E. Newcombe for assisting with sample collection, E. Laman-Trip, J. Choat, M. McCormick, and M. Meekan for their advice on otolith analysis and settlement marks, J. Murray and two anonymous referees for commenting on the manuscript, T. Hickey for his advice on population connectivity in New Zealand triplefins, M. Wellenreuther for her comments regarding New Zealand triplefins. This work was made possible with financial support from a Marsden Grant of the Royal Society of New Zealand to KDC.

References

Aldanondo N, Cotano U, Etxebeste E, Irigoien X, Álvarez P, Martínez de Murguía A, Herrero DL (2008) Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. Fish Res 93:257–264

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP (2007) Local replenishment of coral reef fish population in a marine reserve. Science 316:742–744

Ammann AJ (2004) SMURFs: standard monitoring units for the recruitment of temperate reef fishes. J Exp Mar Biol Ecol 299:135–154

Atema J, Kingsford MJ, Gerlach G (2002) Larval reef fish could use odour for detection, retention and orientation to reefs. Mar Ecol Prog Ser 241:151–160

Bay LK, Crozier RH, Caley MJ (2006) The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar Biol 149:1247–1256

Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR (2006) Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar Biol 149:899–913

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE (2008) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc R Soc B 275:1803–1809

Brogan MW (1994) Distribution and retention of larval fishes near reefs in the Gulf of California. Mar Ecol Prog Ser 115:1–13

Brothers EB, Williams DA, Sale PF (1983) Length of larval life in 12 families of fishes at "one tree lagoon", Great Barrier Reef, Australia. Mar Biol 76:319–324

Campana SE (1992) Measurement and interpretation of the microstructure of fish otoliths. In: Stevenson DK, Campana SE (eds) Otoliths microstructure examination and analysis. Can Spec Publ Fish Aquat Sci 117: 59–71

Carreras-Carbonell J, Macpherson E, Pascual M (2006) Population structure within and between subspecies of the Mediterranean triplefin fish *Tripterygion delaisi* revealed by highly polymorphic microsattelite loci. Mol Ecol 15:3527–3539

Choat JH (2006) Phylogeography and reef fishes: bringing ecology back into the argument. J Biogeogr 33:967–968

Clark DL, Leis JM, Hay AC, Trnski T (2005) Swimming ontogeny of larvae of four temperate marine fishes. Mar Ecol Prog Ser 292:287–300

Connell SD, Jones GP (1991) The influence of habitat complexity on postrecruitment processes in a temperate reef fish population. J Exp Mar Biol Ecol 151:271–294

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Annu Rev Mater Sci 1:443–466

Doherty PJ, Planes S, Mather P (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. Ecology 76:2373–2391

Døving KB, Stabell OB, Östlund-nillson S, Fisher R (2006) Site fidelity and homing in tropical coral reef cardinalfish: are they using olfactory cues? Chem Senses 31:265–272

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Feary DA, Clements KD (2006) Habitat use by triplefin species (Tripterygiidae) on rocky reefs in New Zealand. J Fish Biol 69:1031–1046

Feary DA, Wellenreuther M, Clements KD (2009) Trophic ecology of New Zealand triplefin fishes (Family Tripterygiidae). Mar Biol 156:1703–1714

Fisher R (2005) Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. Mar Ecol Prog Ser 285:223–232

Fowler AJ (1989) Description, interpretation and use of the microstructure of otoliths from juvenile butterflyfishes (Family Chaetodontidae). Mar Biol 102:167–181

Francis MP (2001) Coastal fishes of New Zealand: an identification guide, 3rd edn. Reed Books, Auckland

Fricke R (1994) Tripterygiid fishes of Australia, New Zealand and the southwest Pacific Ocean (Teleostei). Koeltz Scientific, Konigstein

Fuiman LA (2002) Special considerations of fish eggs and larvae. In: Fuiman LA, Werner RG (eds) Fishery science: the contributions of early life stages. Blackwell Science, Oxford, pp 1–32

Galarza JA, Carreras-Carbonell J, Macpherson E, Pascual M, Roques S, Turner GF, Rico C (2009) The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. Proc Natl Acad Sci USA 106:1473–1478

Geffen AJ (1992) Validation of otolith increment deposition rate. In: Stevenson DK, Campana SE (eds) Otolith microstructure examination and analysis. Can Spec Publ Fish Aquat Sci 117: 101–113

Gerlach G, Atema J, Kingsford MJ, Black KP, Miller-Sims V (2007) Smelling home can prevent dispersal of reef fish larvae. Proc Natl Acad Sci USA 104:858–863

Giovannotti M, La Mesa M, Caputo V (2009) Life style and genetic variation in teleosts: the case of pelagic (*Aphia minuta*) and benthic (*Gobius niger*) gobies (Perciformes: Gobiidae). Mar Biol 156:239–252

Griffiths SP (2000) The use of clove oil as an anaesthetic and method for sampling intertidal rockpool fishes. J Fish Biol 57:1453–1464

Hardy GS (1987) Descriptions of a new genus and two new species of tripterygiid fishes from New Zealand. Natl Mus NZ Rec 3:47–58

Helfman GS, Collete BB, Facey DE, Bowen BW (2009) The diversity of fishes. Wiley-Blackwell, Oxford

Hernaman V, Munday PL, Schläppy ML (2000) Validation of otolith growth-increment periodicity in tropical gobies. Mar Biol 137:715–726

Hickey AJR, Clements KD (2003) Key metabolic enzymes and muscle structure in triplefin fishes (Tripterygiidae): a phylogenetic comparison. J Comp Physiol B 173:113–123

Hickey AJR, Lavery SD, Eyton SR, Clements KD (2004) Verifying invasive marine fish species using molecular techniques: a model example using triplefin fishes (family Tripterygiidae). NZ J Mar Freshw Res 38:439–446

Hickey AJR, Lavery SD, Hannan DA, Baker S, Clements KD (2009) New Zealand triplefin fishes (Family Tripterygiidae): contrasting population structure and mtDNA diversity within a marine species flock. Mol Ecol 18:680–696

Hickford MJH, Schiel DR (2003) Comparative dispersal of larvae from demersal versus pelagic spawning fishes. Mar Ecol Prog Ser 252:255–271

Hilton Z, Wellenreuther M, Clements KD (2008) Physiology underpins habitat partitioning in a sympatric sister-species pair of intertidal fishes. Funct Ecol 22:1108–1117

Jenkins GP, May HM (1994) Variations in settlement and larval duration of King George whiting, *Sillaginodes punctata* (Sillanginidae), in Swan Bay, Victoria, Australia. Bull Mar Sci 54:281–296

Job SD, Bellwood DR (2000) Light sensitivity in larval fishes: implications for vertical zonation in the pelagic zone. Limnol Oceanogr 45:362–371

Kingsford MJ (1992) Drift algae and small fish in coastal waters of northeastern New Zealand. Mar Ecol Prog Ser 80:41–54

Kingsford MJ, Choat JH (1989) Horizontal distribution patterns of presettlement reef fish: are they influenced by the proximity of reefs? Mar Biol 101:285–298

Leis JM, Carson-Ewart BM (1997) Swimming speeds of the late larvae of some coral reef fishes. Mar Ecol Prog Ser 159:165–174

Leis JM, Hay AC, Howarth GJ (2009) Ontogeny of in situ behaviours relevant to dispersal and population connectivity in larvae of coral-reef fishes. Mar Ecol Prog Ser 379:163–179

Leis JM, Hay AC, Trnski T (2006) In situ ontogeny of behaviour in pelagic larvae of three temperate marine, demersal fishes. Mar Biol 148:655–669

Longenecker K, Langston R (2005) Life history of the Hawaiian blackhead triplefin, *Enneapterygius atriceps* (Blennioidei, Tripterygiidae). Environ Biol Fish 73:243–251

López-Sanz À, Vert N, Zabala M, Sabatés A (2009) Small-scale distribution of fish larvae around the Medes Islands marine protected area (NW Mediterranean). J Plankton Res 31:763–775

Macpherson E, Raventos N (2006) Relationship between pelagic larval duration and geographic distribution of Mediterranean littoral fishes. Mar Ecol Prog Ser 327:257–265

McCormick MI (1999) Delayed metamorphosis of a tropical reef fish (*Acanthurus triostegus*): a field experiment. Mar Ecol Prog Ser 176:25–38

McDermott CJ, Shima JS (2006) Ontogenetic shifts in microhabitat preference of the temperate reef fish *Forsterygion lapillum:* implications for population limitation. Mar Ecol Prog Ser 320:259–266

McLay CL (1976) An inventory of the status and origin of New Zealand estuarine systems. Proc NZ Ecol Soc 23:8–26

Morales-Nin B (2000) Review of the growth regulation processes of otolith daily increment formation. Fish Res 46:53–67

Nelson JS (2006) Fishes of the world, 4th edn. John Wiley and Sons, New Jersey

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proc Natl Acad Sci USA 104:1266–1271

Patzner RA, Hastings PA, Springer VG, Wirtz P, Gonçalves EJ (2009) List of valid species of blennies. In: Patzner RA, Gonçalves EJ, Hastings PA, Kapoor BG (eds) The biology of blennies. Science Publishers, Enfield, pp 443–473

Paulin CD, Roberts C (1992) The rockpool fishes of New Zealand. Museum of New Zealand, Wellington

Pineda J, Hare JA, Sponaugle S (2007) Larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography 20:22–39

Purcell JFH, Cowen RK, Huges CR, Williams DA (2006) Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. Proc R Soc Lond B 272:1483–1490

Raventós N, Macpherson E (2001) Planktonic larval duration and settlement marks on the otoliths of Mediterranean littoral fishes. Mar Biol 138:1115–1120

Riginos C, Victor BC (2001) Larval spatial distributions and other early life-history characteristics predict genetic differentiation in eastern Pacific blennioid fishes. Proc R Soc Lond B 268:1931–1936

Rocha LA, Robertson DR, Roman J, Bowen BW (2005) Ecological speciation in tropical reef fishes. Proc R Soc Lond B 272:573–579

Russell BC (1983) The food and feeding habits of rocky reef fish of north-eastern New Zealand. NZ J Mar Freshw Res 17:121–145

Sabatés A, Zabala M, García-Rubies A (2003) Larval fish communities in the Medes Islands Marine reserve (Northwest Mediterranean). J Plankton Res 25:1035–1046

Shima JS, Swearer SE (2009) Larval quality is shaped by matrix effects: implications for connectivity in a marine metapopulation. Ecology 90:1255–1267

Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. Evolution 47:264–279

Subedar K (2009) Homing in two New Zealand triplefins. Unpublished MSc thesis, University of Auckland

Syms C (1995) Multi-scale analysis of habitat association in a guild of blennioid fishes. Mar Ecol Prog Ser 125:31–43

Thompson SM (1979) Ecological and behavioural factors influencing the distribution and abundance patterns of tripterygiid fishes with particular reference to *Tripterygion varium.* Unpublished MSc thesis, University of Auckland

Tolimieri N, Jeffs A, Montgomery JC (2000) Ambient sound as a cue for navigation by the pelagic larvae of reef fishes. Mar Ecol Prog Ser 207:219–224

Tricklebank KA, Jacoby CA, Montgomery JC (1992) Composition, distribution and abundance of neustonic ichthyoplankton off Northeastern New Zealand. Estuar Coast Shelf Sci 34:263–275

Vallès H, Kramer DL, Hunte W (2008) Temporal and spatial patterns in the recruitment of coral-reef fishes in Barbados. Mar Ecol Prog Ser 363:257–272

Victor BC (1986a) Duration of the planktonic larval stage of 100 species of Pacific and Atlantic wrasses (Family Labridae). Mar Biol 90:317–326

Victor BC (1986b) Delayed metamorphosis with reduced larval growth in a coral reef fish *Thalassoma bifasciatum*. Can J Fish Aquat Sci 43:1208–1213

Victor BC, Wellington GM (2000) Endemism and the pelagic larval duration of reef fishes in the eastern Pacific Ocean. Mar Ecol Prog Ser 205:241–248

Walker SPW, McCormick MI (2004) Otolith-check formation and accelerated growth associated with sex change in an annual protogynous tropical fish. Mar Ecol Prog Ser 266:201–212

Wellenreuther M, Barrett PT, Clements KD (2007) Ecological diversification in habitat use by subtidal triplefin fishes (Tripterygiidae). Mar Ecol Prog Ser 330:235–246

Wellenreuther M, Barrett PT, Clements KD (2009) The evolution of habitat specialisation in a group of marine triplefin species. Evol Ecol 23:557–568

Wellenreuther M, Clements KD (2007) Reproductive isolation in temperate reef fishes. Mar Biol 152:619–630

Wellenreuther M, Syms C, Clements KD (2008) Consistent spatial pattern across biogeographic gradients in temperate reef fishes. Ecography 31:84–94

Wellington GM, Victor BC (1989) Planktonic larval duration of 100 species of Pacific and Atlantic damselfishes (Pomacentridae). Mar Biol 101:557–567

Willis TJ, Roberts CD (1996) Recolonisation and recruitment of fishes to intertidal rockpools at Wellington, New Zealand. Environ Biol Fish 47:329–343

Wilson DT, McCormick MI (1997) Spatial and temporal validation of settlement-marks in the otoliths of tropical reef fishes. Mar Ecol Prog Ser 153:259–271

Wilson DT, McCormick MI (1999) Microstructure of settlement-marks in the otoliths of tropical reef fishes. Mar Biol 134:29–41