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Comparative Morphology of the Mechanosensory Lateral Line System in a Clade of New Zealand Triplefin Fishes

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Key Words
Eco-morphology · Interspecific divergence · Lateral line · Mechanosensory · Neuromasts · Teleost · Tripterygiidae

Abstract
The mechanoreceptive lateral line system in fishes detects hydrodynamic stimuli and plays a critical role in many fundamental behaviours, including orientation to water currents and the detection of stationary objects, prey and predators. Interspecific variation in lateral line structure may result from a process of functional adaptation, with the background level of hydrodynamic activity proposed as an important selective pressure. Here we use the eight species of the ecologically diverse New Zealand marine triplefin fish of the genus *Forsterygion* and one species from the sister genus *Notoclinops* to investigate interspecific differences in lateral line morphology and to assess the relationship between lateral line characteristics and exposure to wave energy (fetch/depth ratio). Overall, the results show that lateral line traits are divergent between species, and these differences could in part be related to the wave exposure of the habitats that the species occupy. Specifically, numbers of canal neuromasts differed significantly between species, and most canal groupings increased in neuromast number with fetch/depth ratio, while the number and area of some superficial neuromast groupings decreased significantly with exposure. Distribution of superficial neuromasts along the trunk in the semi-pelagic and paedomorphic species *F. maryannae* differed from the other, demersal species, which may be associated with the unique lifestyle of this species and/or developmental processes. Canal architecture also differed considerably between species, but displayed no relationship with fetch/depth ratio. The results from this study indicate that some interspecific differences in lateral line organs may be a by-product of selection for habitat divergence. Future work should explore additional causal factors that might have influenced the evolution of lateral morphology in these species, including phylogenetic and allometric effects.

Introduction

The mechanosensory lateral line system is a hydrodynamic sense that is ubiquitous among fishes and aquatic amphibians. The lateral line system comprises an array of sensory structures called neuromasts, which detect water movement relative to the fish, and is thus akin to a sense of 'touch at a distance' [Dijkgraaf, 1963]. Neuromasts are comprised of sensory hair and support cells that are covered by gelatinous cupula [Montgomery et al.,

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1995; Webb, 1989]. They can be located either superficially on the skin (superficial neuromasts) or recessed within fluid-filled subepidermal canals (canal neuromasts). In fishes, the lateral line system comprises many sense organs which are typically present on the trunk as well as on the head [Coombs et al., 1988; Webb, 1989]. The ability of the lateral line system to encode hydrodynamic information plays a critical role in many fundamental behaviours, such as the detection of stationary objects [Weissert and Campenhausen, 1981; Windsor et al., 2008], prey [Janssen, 1996; Montgomery and Milton, 1993; Montgomery et al., 2002] and predators [Pohlmann et al., 2004], and is also used for rheotaxis [Baker and Montgomery, 1999; Kanter and Coombs, 2003; Montgomery et al., 1997].

Ecomorphological studies of lateral line systems predict that closely related fish species that occupy distinct habitat niches should display differences in lateral line systems as a result of divergent selection regimes in their respective habitats [Coombs et al., 1992; Dijkgraaf, 1962; Montgomery et al., 1994; Vischer, 1990]. More specifically, it has been suggested that variation in lateral line systems between ecologically divergent species can be caused by a process of functional adaptation, with the main selective pressure being the level of background hydrodynamic activity [Coombs et al., 1992]. For example, the two components of the lateral line systems – superficial neuromasts and canal neuromasts – have different response properties and can serve different behavioural functions. This hypothesis is further supported by the finding that species that differ in habitat exhibit considerable differences in the morphological characteristics of these types of neuromasts [Coombs et al., 1988; Dijkgraaf, 1962; Vischer, 1990]. Empirical studies have indicated that species inhabiting low-noise environments are commonly characterised by a widened or reduced canal system and a proliferation of superficial neuromasts, while species in high-noise environments show a more complex and narrow canal system, as well as a reduction in the number of superficial neuromasts [Coombs et al., 1988; Dijkgraaf, 1962; Janssen, 1996; Vischer, 1990]. Similarly, pelagic fishes that inhabit turbulent waters possess few superficial neuromasts, but a well-developed system of canal neuromasts [Coombs et al., 1988]. In contrast, bottom dwellers and slow- or intermittently swimming fish often possess many superficial neuromasts with either no or a reduced canal system, which is similar to what has been found in amphibians. In addition, a recent study by Wark and Peichel [2010] showed that the lateral line sensory system of ecologically divergent threespine sticklebacks can vary significantly even between individuals and among populations. These studies suggest that the diversity of lateral line systems between and within species is associated with adaptation to different environments. It should be noted, however, that despite these general observations across taxa, few studies have involved within-clade comparisons or quantitatively measured environmental features. The use of closely related species in conjunction with a defined environmental continuum in this context is important, since this approach allows the possibility of more directly testing the relationships between divergent habitat use and lateral line systems.

The New Zealand triplefin (family Tripterygiidae) fauna consists of 12 genera and 26 endemic species which inhabit intertidal, reef, deep-water and estuarine habitats [Wellenreuther et al., 2007]. This fauna thus represents a significant proportion of triplefin diversity worldwide [Hickey and Clements, 2005; Hickey et al., 2009], making it an excellent model system for comparative studies. Previous work has indicated that there is little specialization in diet, jaw morphology and male breeding colouration [Feary et al., 2009; Wellenreuther and Clements, 2007], but considerable diversification in physiology [Brix et al., 1999; Hickey and Clements, 2003; Hilton et al., 2008] and habitat [Feary and Clements, 2006; Syms, 1995; Wellenreuther et al., 2007]. Diet appears to be mainly associated with habitat choice or size-dependent feeding behaviour. The majority of species are characterised by a jaw apparatus consistent with a relatively high velocity, low force jaw movement indicative of a diet of evasive prey [Feary et al., 2009]. Despite the lack in trophic diversification, all 26 species have diverged considerably in habitat use [Wellenreuther et al., 2007, 2008], and there is evidence that this habitat diversification is linked with physiological performance [haemoglobin components in relation to habitat depth: Brix et al., 1999; oxygen consumption, ventilation frequency and hypoxia tolerance in relation to rockpool height: Hilton et al., 2008], suggesting that selection has contributed to the evolutionary divergence of species. The use of habitats appears to be the result of active habitat choice exhibited by larvae at settlement [Wellenreuther and Clements, 2008] and habitat-driven assortative mating behaviour, which together decrease ecological overlap and promote reproductive isolation [Wellenreuther and Clements, 2007].

Of the 12 genera within the New Zealand fauna, the genus *Forsterygion* contains the most species (see phylogeny in fig. 1). *Forsterygion* species occupy a wide range of habitats, ranging from highly wave-exposed reefs to shel-

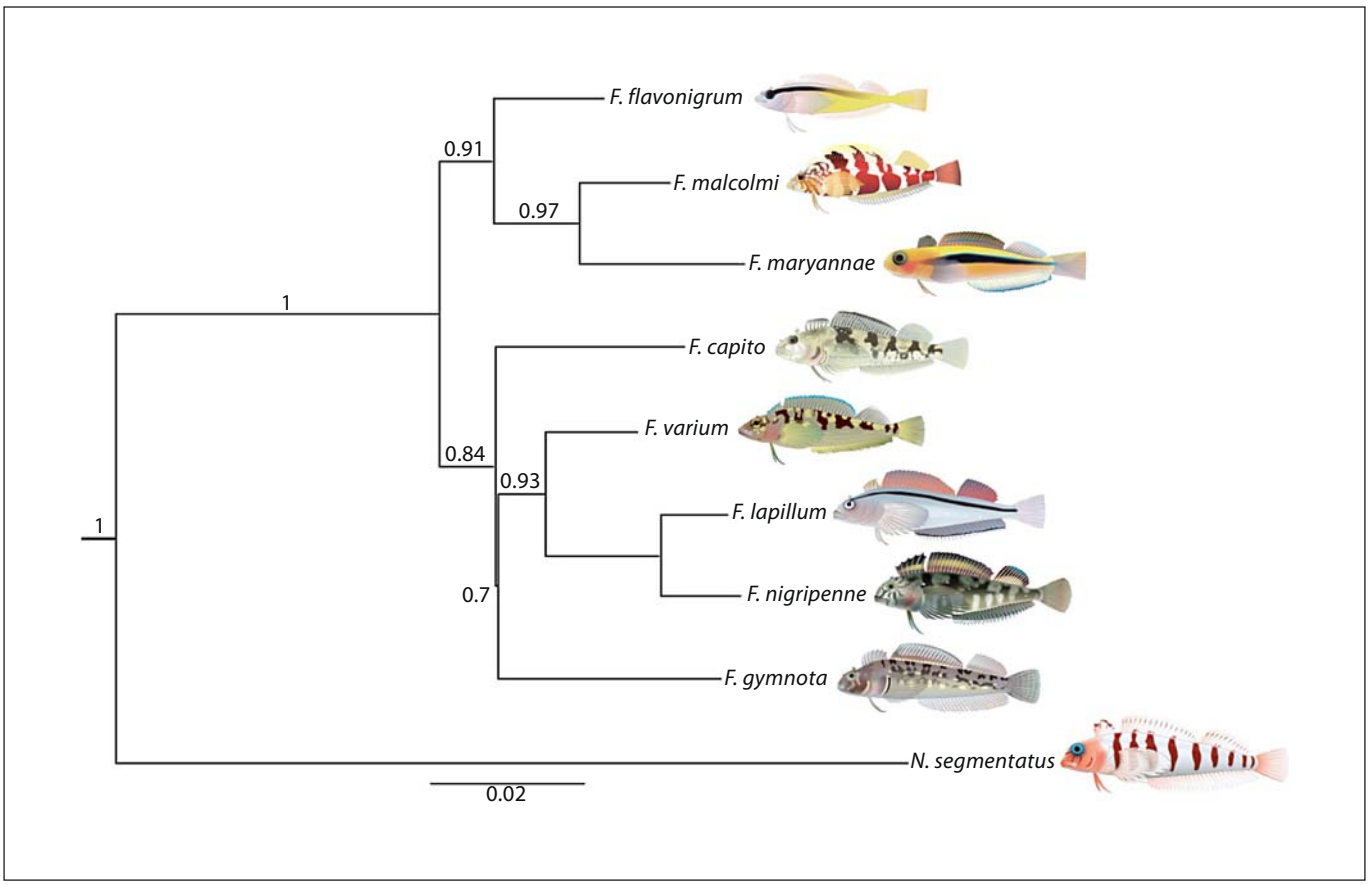

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Fig. 1. Phylogeny of the genus *Forsterygion*, with *Notoclinops segmentatus* as an outgroup, following the topology of Hickey and Clements [2005]. The tree is based on a Bayesian analysis of sequence data from three mitochondrial genes (12S, 16S and control region) and the nuclear gene (ETS2). Values associated with branches are Bayesian posterior probabilities.

tered and shallow bays [Clements, 2003; Feary and Clements, 2006; Syms, 1995; Syms and Jones, 1999; Wellenreuther et al., 2007]. A total of eight species comprise the genus *Forsterygion* [Jawad, 2008], all of which are endemic to New Zealand, although three have been introduced to Australia [Hickey et al., 2004]. The high diversity of habitat use in this genus is exemplified by two species that are found in habitats that are unique among all triplefin species worldwide. *Forsterygion nigripenne* is the only estuarine triplefin species, while *F. maryannae* is the only planktivorous and semi-pelagic triplefin species [Clements, 2003].

The present study describes interspecific variation in the lateral line system of all eight species in the genus *Forsterygion*, and for comparative purposes, the lateral line system of a species belonging to the closely related genus *Notoclinops*. Morphological traits were subsequently correlated to the wave energy exposure of each species' habitat to examine whether the morphological features of the lateral line system, such as the relative abundance of superficial and canal neuromasts, are correlated with ambient levels of hydrodynamic noise. An understanding of these ecomorphological relationships is a prerequisite for functional research exploring the diversity of the lateral line system within and between species.

Materials and Methods

Study Species, Wave Exposure Calculations and Fish Collections

Triplefin fishes belong to the family Tripterygiidae, which is one of six families in the suborder Blennioidei. Descriptive work by Fricke [1994] showed that the lateral line morphology along the trunk canal of triplefin fishes from New Zealand and Australia

and the Southwest Pacific is characterised by either a single continuous lateral line (4–56 tubular pored scales) or a double and discontinuous lateral line with an anterior series of 6–38 tubular pored scales and a posterior series with 0–38 notched scales. In addition, Fricke [1994] found further that the lateral line canals of the suborbital ring are typically covered by bone. A recent revision of New Zealand triplefin genus *Forsterygion* led to the inclusion of four species formerly placed elsewhere [Jawad, 2008]. *Forsterygion capito*, *F. gymnota* and *F. nigripenne* were previously placed in the genus *Grahamina,* while the fourth species, *F. maryannae,* was previously placed in the monotypic *Obliquichthys*. The revised *Forsterygion* now consists of eight species: *F. capito*, *F. flavonigrum*, *F. gymnota, F. lapillum, F. malcolmi*, *F. maryannae, F. nigripenne* and *F. varium* [Jawad, 2008]. All *Forsterygion* species are endemic to New Zealand, although *F. varium*, *F. gymnota* and *F. lapillum* have been introduced to Australia [Hickey et al., 2004]. A discontinuous lateral line with both notched and tubular scales was used as a diagnostic character in Fricke's [1994] diagnosis of the genus *Forsterygion*. *Grahamina* was diagnosed with a discontinuous lateral line [Fricke and Roberts, 1993]. The monotypic genus *Obliquichthys* was diagnosed with a lateral line that consists of 21 (18–22) tubular scales only, shallowly arched along its length, extending for about two-thirds the length of the second dorsal fin [Hardy, 1987].

In broad terms, the habitat use of *Forsterygion* species can be characterised as follows: *Forsterygion capito* and *F. nigripenne* are typically found in muddy and shallow sheltered habitats (the latter species is estuarine), *F. varium*, *F. malcolmi* and *F. flavonigrum* are found in exposed, subtidal rocky habitats, *F. lapillum* is found in intertidal and shallow subtidal habitats with cobbles and small rocks, and *F. gymnota* is found in exposed, turbid coastal habitats [Feary and Clements, 2006; Syms, 1995; Wellenreuther et al., 2007]. These seven species all share a benthic lifestyle and prey on a wide range of small sessile to mobile benthic invertebrates, including amphipods, archaeogastropods, barnacle cirri, ophiuroids and errant polychaetes [Feary et al., 2009]. *F. maryannae* differs from the previous seven species in that individuals have a semi-pelagic lifestyle and school in groups of 10–200 individuals in medium depths that are moderately exposed [Wellenreuther et al., 2007], with individuals using the benthos only when resting [Syms and Jones, 1999]. All species are diurnally active. As juveniles and adults, they provide food for predatory fish species including eels, John Dory, scorpionfish, sea perch, goatfish and blue cod [e.g. Mutch, 1983]. Physiological investigation of the key metabolic enzymes and muscle structure of New Zealand triplefin species demonstrated that *F. maryannae* is paedomorphic and retains a larval muscle architecture, which is thought to increase the aerobic potential for sustained swimming [Hickey and Clements, 2003]. The unique semi-pelagic lifestyle of *F. maryannae* is coupled with a distinct diet, since this species is the only explicitly planktivorous triplefin species [Feary et al., 2009]. *Notoclinops segmentatus* was included for comparative purposes for two reasons. First, *Notoclinops* is the sister group to *Forsterygion* [Hickey and Clements, 2005; Hickey et al., 2009], and second, *N. segmentatus* is the most common *Notoclinops* species in coastal New Zealand waters and is thus easily collected and studied [Wellenreuther et al., 2007].

The lateral line could serve several functions in the study triplefin species. For example, all triplefin species included in our study predate on mobile, invertebrate prey [Feary et al., 2009]. Hence, the lateral line system in these species could serve prey detection and capture. Furthermore, the lateral line might also facilitate predator detection, since triplefins are predated upon by large carnivorous fish including eels, John Dory, scorpionfish, sea perch, goatfish and blue cod [Clements, 2003; Mutch, 1983]. One species, *F. maryannae*, is a semi-pelagic species, thus the lateral line might also be important for schooling in this species

Only adult triplefin fishes were collected for the comparative morphological work to avoid variation in lateral line system due to ontogenetic effects. Adult fish were selected on the basis of body size using data from earlier work [Wellenreuther and Clements, 2008]. All fishes were either collected on SCUBA with hand nets and slurp guns, or with bait catchers from wharfs between October 2005 and April 2006. Fish collections were conducted at several sites within the Hauraki Gulf area (36°36′0S, 174°50′0E) and Whatipu (37°10′60S, 174°31′00E) in northeastern New Zealand. After capture, fishes were euthanized with an overdose of the anaesthetic clove oil [following recommendations by Griffiths, 2000], measured with callipers to the nearest mm (total length), and then prepared for morphological examination. The capture and handling protocols were performed according to the guidelines of the ethics committee of the University of Auckland.

The relative exposure to water movement of each species' habitat was estimated using quantitative habitat data from Wellenreuther et al. [2007]. They estimated mean exposure of each species via calculation of maximum fetch, i.e. the distance of open water over which waves can be generated by wind, thus making this measure an approximation of wave energy exposure. In short, visual underwater counts of triplefin fishes were conducted at a wide variety of locations along the New Zealand coastline, and for each count, a location fix was taken using a handheld Garmin® 12 global positioning system. Geographic coordinates for each individual fish were entered into the program Fetch Effect Analysis, which measures fetch distance for each 20° sector on a compass rose from a given point [Wellenreuther et al., 2007]. This measure does not account, however, for the fact that shallow-living species will be more affected by wave exposure than species inhabiting deep waters. For this reason, to obtain a classification that takes into account the depth of the habitat that a species occupies, the mean fetch was divided by the mean depth of the habitat of each species. This correction resulted in a relative exposure measure for each species that could be used as a proxy for wave exposure. It should be noted, however, that our measure of wave energy exposure does not take into account tidal currents, the size and shape of bottom structures (e.g. substratum, ripples, and rocks) and flow rates (e.g. due to wind direction and gradient). Nevertheless, our wave exposure estimate was based on a total of 12,375 individual fish observations (mean per species: 1,375), thus providing a robust relative measure of species-specific exposure to hydrodynamic noise.

Morphological Procedures and Measurements

Canal Systems, Pore Numbers and Canal Dimensions

For measurements of the lateral line canal system, pore numbers and canal dimensions, fish specimens were fixed in 10% formalin for a minimum of 48 h and then placed in 70% ethanol. To identify the lateral line canal system and the number of canal pores, a minimum of six specimens from each species were placed in 100% ethanol for 2 h, followed by submersion in ethanol to dehydrate and make the bodies translucent. To visualise the lateral

line canal system, water was removed from the fixed specimens by tapping a piece of absorbent cotton over the lateral line canals, and then an Indian ink was injected into the canal pores of each specimen (with a syringe). Following the staining with Indian ink, photographs of a minimum of five specimens of each species were taken using a Canon G1 camera. The lateral line canals were classified following the terminology proposed by Fricke [1994] and Coombs et al. [1998]. To quantify the number of canal pores in each lateral line canal system on the head (cranial pores), each canal system was identified and the pores were counted on the right side of the head.

To measure the canal dimensions of the preopercular-mandibular and trunk canal, canals were dissected with a scalpel in at least three specimens of each species. The canal sections were then placed in Calex for 2–3 h and then prepared for standard paraffin histology. Ten transverse sections 7 μm wide were cut with a Microm HM330 Rotary microtome through the canals of each specimen. These sections were stained with Ehrich's eosin and hematoxylin and then photographed under a compound microscope (Leica, DMR Stereozoom microscope; Leica-Heerbrugg, Heerbrugg, Switzerland). The following dimensions were recorded: (1) canal cross-sectional area at the level of a neuromast, (2) cross-sectional area of the canal between neuromasts, and (3) the canal neuromast area. From each photograph, three measurements were made for each dimension and then averaged.

Canal areas were commonly occluded by sensory structures (i.e. by neuromasts, cupulae and associated sensory support cells). To quantify the degree of occlusion, 10 longitudinal sections from both the preopercular-mandibular and trunk canal were cut with a HM330 Rotary microtome at a thickness of 10 μm. Photographs were taken with both a dissecting (Leica, Wild M3C) and compound microscope (Leica, DMR), and for each photograph three measurements were taken and then averaged. All morphological measurements were made using the software ImageJ [Rasband, 1997].

Superficial Neuromasts

To examine the number of, and area occupied by, superficial neuromasts, fishes (n = 6 of each species) were rinsed and fixed in Karnovsky's [1965] formaldehyde-glutaraldehyde fixative (8% paraformaldehyde, 50% gultaraldehyde, 0.2 M Sorensen's phosphate buffer) for a period of four or more days. Specimens were sectioned with a scalpel posterior to the pectoral fin insertion and anterior to the caudal fin and then cut longitudinally. The tissue was dehydrated in a graded series of ethanol (5, 15, 30, 50, 70, 90 and 100% for 25 min), followed by a second dose of 100% ethanol for 25 min. The tissue was then critical-point dried and mounted onto alloy stubs and stored in desiccation jars. Finally, the tissues were sputter-coated with gold for examination under the field-emission scanning electron microscope (SEM, FEI Quanta XL30). As fresh specimens could not be collected across the whole range of species, vital staining may have led to inconsistent results between preserved and fresh specimens. SEM was deemed to be more appropriate for avoiding bias in counting superficial neuromasts.

The superficial neuromast groupings were named following the terminology outlined in Carton and Montgomery [2004], which is an extension of the terminology proposed by Coombs et al. [1988]. Five cranial groupings of superficial neuromasts were identified, namely the infraorbital (equivalent to the ventral infraorbital line), postocular (equivalent to the dorsal supraorbital line), cheek, opercular, and the antorbital grouping (equivalent to the rostral supraorbital and rostral infraorbital line). All these groupings or their equivalents are thoroughly described in Carton and Montgomery [1994, fig. 5], with the exception of the opercular grouping, which is described in Coombs et al. [1988]. Photomicrographs of the superficial neuromasts from the five distinct cranial groupings and the trunk region were captured digitally. The number of superficial neuromasts belonging to each grouping was mapped and the total area occupied by superficial neuromasts (area covered by hair and mantle cells) was measured. A minimum of three counts and area measurements of each neuromast grouping was taken for each species. Area measurements were made using ImageJ [Rasband, 1997]. Photomicrographs with a curvature of <45% were corrected using the cosine function [Coombs and Montgomery, 1992], while photomicrographs with a curvature of >45% were excluded from this study. The fragile nature of the structure under investigation meant that it was often not possible to conduct all measurements of the superficial neuromast groupings on the same specimens for a species, and thus different specimens had to be used sometimes. One source of error in the SEM measurements included fixation shrinkage, which has been estimated to be around 10% [Janssen et al., 1987]. We assumed that fixation shrinkage affected the tissues of all species in a non-systematic way, and would thus lead to a consistent underestimation of neuromast dimensions in all species.

Statistical Analyses

Morphological variables that were skewed or showed bivariate non-linearities were square-root transformed to decouple variance-mean relationships and improve linearity. A general linear model (GLM) was used to investigate the relationship between pore numbers and species, with the pore numbers for the six canal systems as the dependent variable and fish species as the categorical predictor. To examine interspecific differences in canal dimensions, a GLM was run, with species as the categorical predictors and the canal cross-sectional dimensions (area at the level of a neuromast, between neuromast positions within a canal, and the canal neuromast area) as dependent variables. The degree of occlusion of the preopercular-mandibular and trunk canals was investigated using a GLM with the percentage of occlusion as the dependent variable and species membership as the categorical predictor. To compare the number of superficial neuromasts on the five cranial groupings (antorbital, cheek, opercular, postocular and infraorbital grouping) and the trunk grouping among species, a GLM with the number of superficial neuromast per grouping was used as the dependent variable and species as the categorical predictor. To investigate interspecific differences in the superficial neuromast area among species, the superficial neuromast areas of the five cranial groupings and the trunk canal grouping were used as dependent variables and species as a categorical factor in a GLM framework. The density of superficial neuromasts was calculated by dividing the number of superficial neuromasts by the area occupied. All significant posterior differences between species were obtained using Tukey's honestly significantly different post hoc tests, and homogenous groupings were formed based on the output (with the same letter denoting a homogenous group). To examine if the level of habitat-related wave exposure of each species was related to the characteristics of the lateral line system, linear regression coefficients were calculated for the individual morphological traits (see table 1). The

Table 1. Estimated fetch/depth ratio of each species calculated by dividing the mean wave energy exposure (fetch) value of each species by the mean depth use

Species	Fetch/depth ratio
F. nigripenne	0.062
F. flavonigrum	0.204
F. capito	0.212
F. malcolmi	0.638
F. maryannae[a]	0.668
N. segmentatus	0.748
F. lapillum	0.885
F. varium	1.021
F. gymnota	1.345

[a] *F. maryannae* is the only semi-pelagic triplefin species in the world. All other species are bottom dwellers.

Quantitative measures taken from Wellenreuther et al. [2007].

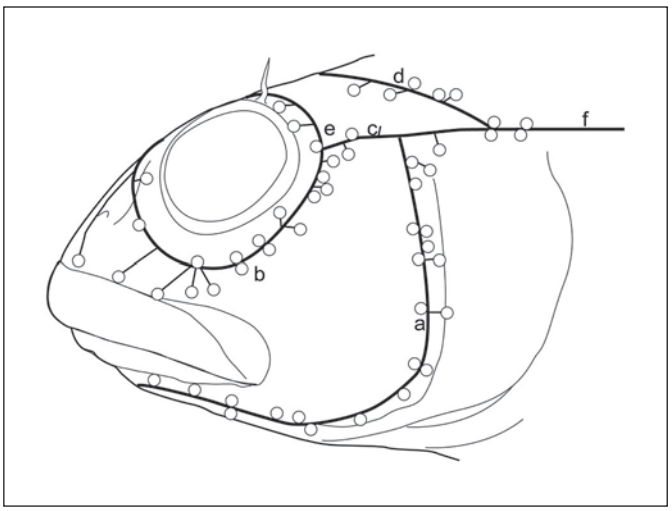


Fig. 2. Schematic view of the bilateral anterior canal system of *F. nigripenne* (note that canal systems were similar across species, and that *F. nigripenne* was chosen as a representative species). Circles indicate pore openings. Canals are labelled as follows: a = preopercular-mandibular, b = infraorbital, c = postocular, d = supratemporal, e = supraorbital, f = trunk.

Bonferroni correction (whereby the threshold for significance is simply reduced by the number of comparisons made) has been criticized for being too stringent and conservative, and there is little consensus when it should be applied [Perneger, 1998]. For these reasons, we chose to follow the recommendation of Perneger [1998] and Nakagawa [2004] and report the unadjusted p values. All data analyses were carried out in Statistica (version 8.0; StatSoft Inc., Tulsa, Okla., USA).

Results

Characterisation of Habitat with Reference to Wave Exposure

The fetch/depth ratio estimates calculated from the data of Wellenreuther et al. [2007] are given in table 1. Low relative wave exposure estimates indicate that species inhabit sheltered environments, while large values denote species that occupy habitats that are frequently wave exposed. With the exception of *F. gymnota*, the habitat sample size used to generate the wave exposure estimates for each species was large, ranging from a total of 337 to 3,803 independent fetch/depth ratio estimates (mean sample size 1,543). The sample size for *F. gymnota* was only 35, and consequently, the resulting wave energy exposure classification for this species needs to be interpreted with caution. The results of the wave energy exposure analysis showed that *F. nigripenne*, *F. flavonigrum* and *F. capito* clearly occupy habitats that are little exposed to wave energy, while *F. gymnota*, *F. varium* and *F. lapillum* are typified by habitats that are considerably more exposed to wave energy. Relative to the other species, *F. maryannae*, *F. malcolmi* and *N. segmentatus* occupy habitats that are exposed to intermediate levels of wave energy. It should be noted, however, that *F. maryannae* might experience a greater range of wave energy exposures than other *Forsterygion* species, due to its semi-pelagic lifestyle.

Morphological Comparisons

Visualisations and subsequent comparisons of the anterior lateral line canal patterns showed that all nine species share a similar lateral line canal system structure. The lateral line canal pattern of all species examined consisted of a narrow canal system [Coombs et al., 1988], characterised by ossified canals of uniform diameter, which is typical of most bony fishes [Webb et al., 2008]. In all species, the anterior pattern of the canal system comprised six paired canals, namely the preopercular-mandibular, infraorbital, postocular, supratemporal, supraorbital and trunk canal (see fig. 2 for a schematic drawing). The cranial bones associated with the canals were identified, and included the dentary, angular and preopercular (preopercular-mandibular), lachrymal and infraorbital series (infraorbital), dermosphenotic (postocular), extrascapular, supraoccipital and epiotic (supratemporal), and frontal and dermosphenotic bones (supraorbital). The left and right lateral trunk canals were joined

Table 2. Means ± standard error of morphological traits examined

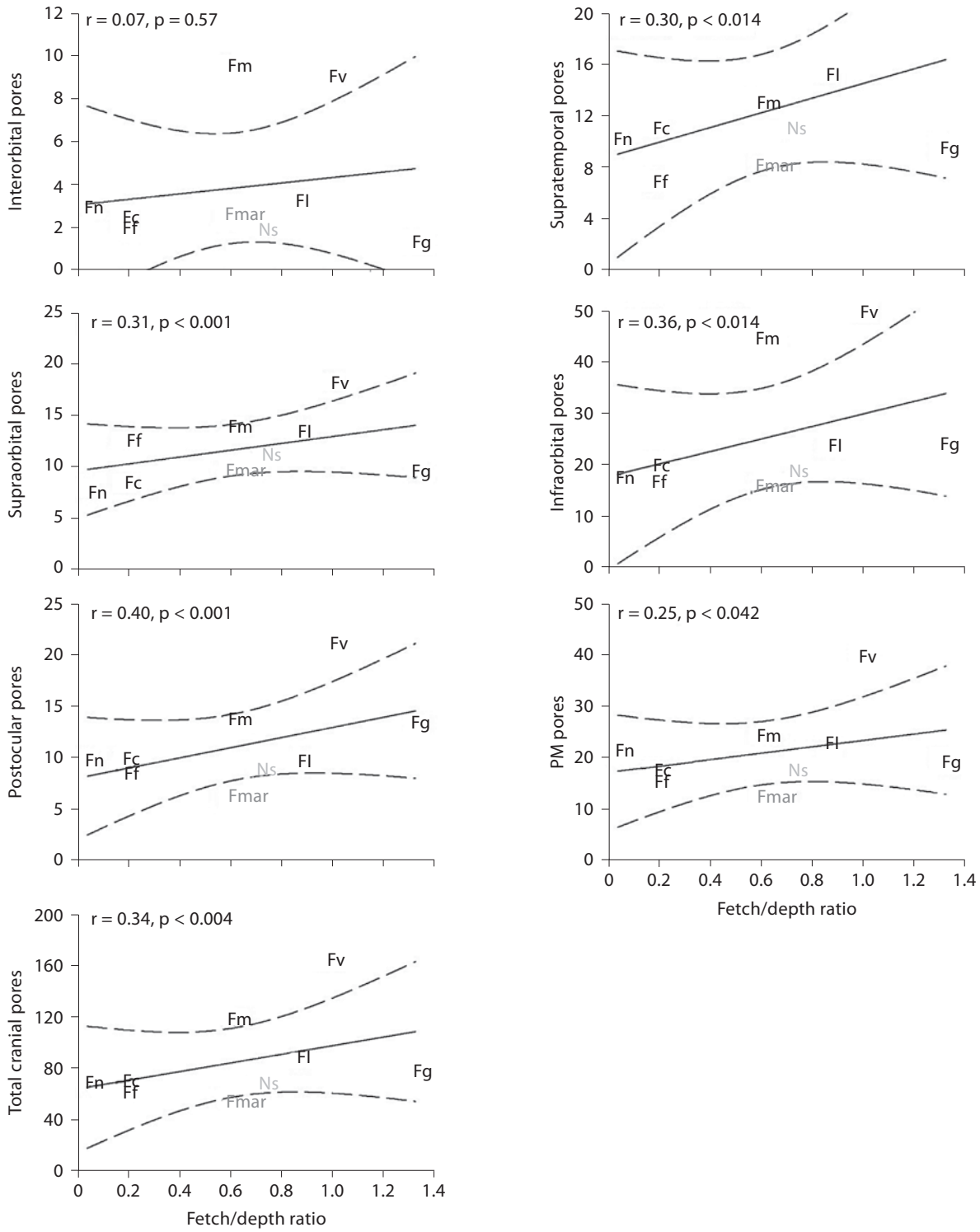
	F. nigripenne	*F. flavonigrum*	*F. capito*	*F. malcolmi*	*F. maryannae*
Number of canal pores on the cranial canal systems ($n \geq 6$ per species, global GLM $F_{48, 269} = 15.78$, $p < 0.0001$)					
Interorbital pores	2.9 ± 0.1^{a}	$2.0 \pm 0^{a, b}$	$2.3 \pm 0.2^{a, b}$	9.5 ± 0.7^{c}	$2.5 \pm 0.2^{a, b}$
Supraorbital pores	7.7 ± 0.3^{b}	$12.5 \pm 0.4^{a, c}$	$8.3 \pm 0.9^{b, c}$	$13.8 \pm 1.5^{a, e}$	$9.5 \pm 0.4^{a, b, c}$
Postocular pores	$9.6 \pm 0.5^{a, b}$	8.5 ± 0.4^{a}	$9.5 \pm 0.6^{a, b, c}$	$13.5 \pm 0.9^{b, c}$	6.0 ± 0.4^{a}
Supratemporal pores	$10.1 \pm 0.6^{a, b}$	6.3 ± 0.6^{a}	$11.0 \pm 0.5^{a, b, c}$	$13.0 \pm 0.7^{b, c}$	$8.2 \pm 0.5^{a, b}$
Infraorbital pores	$17.5 \pm 0.7^{a, b}$	16.7 ± 0.7^{a}	19.8 ± 0.3^{a}	44.8 ± 2.5^{c}	15.8 ± 0.4^{a}
PM pores	$20.9 \pm 0.7^{b, d, e}$	$15.7 \pm 0.2^{a, c}$	$17.8 \pm 0.4^{a, b}$	23.7 ± 1.4^{e}	12.4 ± 0.7^{c}
Total number	$68.7 \pm 1.33^{a, b}$	$62.167 \pm 1.45^{a, b}$	$68.83 \pm 0.40^{a, b}$	118.33 ± 3.72^{d}	54.33 ± 2.12^{a}
Canal cross-sectional areas, mm^2 ($n \geq 3$, global GLM $F_{48, 78} = 3.45$, $p < 0.0001$)					
PM neuromast area (ns)	0.062 ± 0.01	0.041 ± 0.01	0.057 ± 0.01	0.057 ± 0.01	0.043 ± 0.01
PM between neuromasts	$1.395 \pm 0.4^{d, f}$	$0.551 \pm 0.177^{a, b, c}$	$0.863 \pm 0.24^{a, b, c, d, f}$	$1.374 \pm 0.2^{c, d, f}$	$0.551 \pm 0.07^{a, b, c, d}$
PM level of neuromasts	1.654 ± 0.09^{a}	$0.832 \pm 0.001^{a, b}$	$1.716 \pm 0.166^{a, b}$	1.655 ± 0.44^{a}	$0.711 \pm 0.177^{a, b}$
Trunk neuromast area	$0.034 \pm 0.002^{a, b}$	$0.022 \pm 0.009^{a, b}$	$0.029 \pm 0.001^{a, b}$	0.043 ± 0.002^{c}	0.022 ± 0.002^{a}
Trunk between neuromasts	$0.578 \pm 0.1^{a, b}$	$0.73 \pm 0.192^{a, b, c, d}$	$0.681 \pm 0.155^{a, b, c, d}$	1.487 ± 0.1^{d}	$0.459 \pm 0.028^{a, b}$
Trunk level of neuromasts	$0.495 \pm 0.024^{a, b}$	$0.728 \pm 0.136^{a, b, c, d}$	$0.869 \pm 0.159^{a, b, c, d}$	1.135 ± 0.093^{d}	$0.509 \pm 0.061^{a, b}$
Percentage of occlusion ($n \geq 10$ per species, global GLM $F_{16, 44} = 2.89$, $p < 0.003$)					
PM canal (ns)	58.67 ± 1.29	9.33 ± 9.33	13.33 ± 2.33	35.67 ± 2	27 ± 1.62
Trunk canal	$49.67 \pm 4.84^{a, b}$	7.67 ± 7.678^{a}	$22.33 \pm 12.71^{a, b}$	$48.33 \pm 3.67^{a, b}$	$20.33 \pm 11.55^{a, b}$
Number of superficial neuromasts ($n = 6$ per species, global GLM $F_{48, 68} = 6.10$, $p < 0.0001$)					
Infraorbital grouping	$3.66 \pm 0.67^{a, b}$	5 ± 1^{b}	1.67 ± 0.33^{a}	2 ± 0^{a}	1.33 ± 0.33^{a}
Postocular grouping	4.67 ± 0.67^{a}	$3 \pm 0.58^{a, b}$	$3.33 \pm 0.67^{a, b}$		$3.33 \pm 0.88^{a, b}$
Cheek grouping	$2.67 \pm 0.33^{a, b}$	1.33 ± 0.67^{b}	4 ± 0^{a}	$2.33 \pm 0.33^{a, b}$	3.33 ± 0.33^{a}
Opercular grouping	$3 \pm 0^{a, b}$	4 ± 0.58^{b}	$2.33 \pm 0.33^{a, b}$	$2.67 \pm 0.667^{a, b}$	$2.67 \pm 0.33^{a, b}$
Trunk grouping	$25 \pm 1.15^{a, d}$	$16 \pm 2.08^{b, c}$	34 ± 1.15^{e}	$18.67 \pm 1.45^{a, b, c}$	$20.33 \pm 0.88^{a, c}$
Antorbital grouping (ns)	6.67 ± 1.86	8.67 ± 0.88	6.33 ± 0.88	6.33 ± 0.67	6 ± 1
Total number	45.68	38.00	51.68	32.00	37.00
Superficial neuromast areas, μm^2 ($n = 6$ per species, global GLM $F_{8, 98} = 8.80$, $p < 0.0001$)					
Infraorbital grouping	172 ± 9.17^{b}	66.7 ± 7.48^{a}	$102.687 \pm 6.98^{a, b, c}$	151.5 ± 9.02^{b}	$150 \pm 7.64^{b, c}$
Postocular grouping	209.62 ± 20.44^{b}	35 ± 8^{a}	87.13 ± 12.58^{a}		$178.67 \pm 46.33^{a, b}$
Cheek grouping	$226.31 \pm 53.52^{a, b}$	40 ± 5^{a}	87.58 ± 8.9^{a}	341.13 ± 63.65^{b}	343.29 ± 96.6
Opercular grouping	$415.86 \pm 107.93^{a, b}$	$450.2 \pm 128.47^{a, b}$	300.2 ± 32.37^{a}	857.38 ± 199.32^{b}	$465.83 \pm 97.98^{a, b}$
Trunk grouping	$547.38 \pm 97.34^{a, b, c, d}$	$359.63 \pm 40.13^{a, b, c}$	866.18 ± 78.49^{d}	$524 \pm 103.06^{b, c}$	$219.9 \pm 63.462^{a, b}$
Antorbital grouping	$114.3 \pm 4.61^{c, d}$	$52 \pm 4.77^{a, b}$	$60.36 \pm 10.66^{a, b, c}$	$105.33 \pm 14.21^{c, d}$	$104.44 \pm 34.56^{b, c, d}$
Superficial neuromast density, μm^2; calculated as 'number of superficial neuromasts/superficial neuromast area' (see the sections above for statistical results)					
Infraorbital grouping	46.9945	13.34	61.4892	75.75	112.782
Postocular grouping	44.8865	11.6667	26.1652	0	53.6547
Cheek grouping	84.7603	30.0752	21.895	146.4077	103.0901
Opercular grouping	138.62	112.55	128.8412	321.1161	174.4682
Trunk grouping	21.8952	22.4769	25.4759	28.0664	10.8165
Antorbital grouping	17.1364	5.9977	9.5355	16.6398	17.4067

Post hoc test results shown as superscript letters, with species sharing a letter being in the same homogenous group. Species are arranged in order of increasing fetch/depth ratio (from left to right; see table 1). PM denotes the preopercular-mandibular canal.

mid-dorsally by a supratemporal canal that emerged from the temporal segments of the trunk canals. The trunk lateral line had both canal and superficial neuromasts. The trunk canals of all species were discontinuous (disjunct) and terminated in the region beneath the second dorsal fin. Anteriorly, two canals surrounded the eye: an infraorbital canal and a supraorbital canal. Canals also extended onto the cheek and lower jaw. Each of these canals consisted of tubules with pore openings lining the outside of each canal. The pore openings in all species were slightly more elongated and branched near the olfactory nares.

N. segmentatus	*F. lapillum*	*F. varium*	*F. gymnota*
2.0 ± 0[a, b]	3.2 ± 0.4[a]	9.0 ± 0.3[c]	1.4 ± 0.2[b]
11.2 ± 0.9[a, b, c]	13.4 ± 0.5[a]	18.2 ± 3.1[e]	9.7 ± 0.7[a, b, c]
8.5 ± 0.7[a]	9.4 ± 0.4[a]	20.8 ± 2.6[d]	13.2 ± 0.5[c]
11.0 ± 0.3[a, b, c]	15.2 ± 0.8[c]	26.7 ± 3.6[d]	9.5 ± 0.6[a, b]
17.3 ± 0.3[a]	23.6 ± 1.2[b]	49.7 ± 2.6[c]	24.0 ± 0.5[b]
16.8 ± 0.6[a, b, c]	22.2 ± 0.9[d, e]	39.3 ± 2.1[f]	18.9 ± 0.5[a, b, d]
66.83 ± 1.74[a, b]	87 ± 2.8[c]	163.67 ± 12.94[e]	76.7 ± 1.83[b, c]
0.025 ± 0.01	0.041 ± 0.01	0.056 ± 0.01	0.054 ± 0.01
0.498 ± 0.08[a, b]	0.465 ± 0.09[a]	1.672 ± 0.02[f]	1.097 ± 0.14[b, c, d, f]
0.521 ± 0.041[b]	0.715 ± 0.043[a, b]	1.45 ± 0.239[a, b]	1.223 ± 0.226[a, b]
0.011 ± 0.001[a, b]	0.022 ± 0.005[a]	0.034 ± 0.001[a, b]	0.042 ± 0.003[b]
0.703 ± 0.14[a, b]	0.483 ± 0.068[a]	0.746 ± 0.065[c, d]	0.885 ± 0.0789[b, c]
0.399 ± 0.095[a, b]	0.39 ± 0.093[a]	0.941 ± 0.04[c, d]	0.757 ± 0.06[b, c]
32.67 ± 1.66	19.167 ± 7.14	36.67 ± 6.7	54.83 ± 7.76
62.67 ± 8.25[a]	40.8 ± 11.76[a, b]	25.33 ± 1.2[a, b]	52.67 ± 8.84[a]
2.33 ± 0.33[a]	2.33 ± 0.33[a]	2.67 ± 0.33[a, b]	3.33 ± 0.33[a, b]
	3.67 ± 0.88[a]	0.67 ± 0.33[b]	2.67 ± 0.33[a, b]
2.67 ± 0.33[a, b]	2.67 ± 0.67[a, b]		3 ± 0[a, b]
3 ± 0.58[a, b]	2.67 ± 0.33[a, b]	1.33 ± 0.33[a]	1.67 ± 0.33[a]
11 ± 1.15[b]	35 ± 3.05[e]	24 ± 1.15[a, d]	30 ± 1.154[d, e]
5.33 ± 1.2	7 ± 0.58	3.67 ± 1.2	6 ± 0.57
24.33	53.33	32.33	46.68
30.5 ± 5.5[a]	104.63 ± 15.49[a, b, c]	108.4 ± 21.84[a, b, c]	77 ± 13.37[a, c]
	113 ± 8.09[a]	202.5 ± 0[b]	89 ± 14.68[c]
27.8 ± 1.88[b]	37.5 ± 4.97[a]		108.25 ± 18.05[a]
89.67 ± 34.97[a]	261.83 ± 61.15[a]	597.33 ± 147.22[a, b]	118 ± 41.33[a]
102.36 ± 26.01[a]	318 ± 61.54[a, b, c]	666.27 ± 126.71[c, d]	289 ± 44.8[a, b]
30 ± 5.07[a]	59 ± 5.67[a, b, c]	175.4 ± 29.13[d]	45.08 ± 3.01[a, b]
13.0901	44.9056	40.5993	23.1231
0	30.7902	302.2388	33.3333
10.412	14.0449	0	36.0833
29.89	98.0637	449.1203	70.6587
9.3055	9.0857	27.7613	9.6333
5.6285	8.4286	47.7929	7.5133

There were highly significant differences between species in the number of canal pores on cranial canals (supraorbital, postocular, interorbital, infraorbital, supratemporal and preopercular-mandibular canals; overall GLM $F_{48, 269} = 15.78$, $p < 0.0001$; see table 2 for detailed post hoc test results). Follow-up univariate tests showed that the pore numbers differed significantly among species for each of the six canals ($p < 0.001$). Strongest differences in the number of canal pores among species were apparent for the supraorbital and infraorbital canal system, with two species, *F. varium* and *F. malcolmi*, having a significantly greater number of canal pores than all oth-


Interorbital pores
r = 0.07, p = 0.57
Supratemporal pores
r = 0.30, p < 0.014
Supraorbital pores
r = 0.31, p < 0.001
Infraorbital pores
r = 0.36, p < 0.014
Postocular pores
r = 0.40, p < 0.001
PM pores
r = 0.25, p < 0.042
Total cranial pores
r = 0.34, p < 0.004
Fn
Fc
Ff
Fm
Fv
Fl
Fg
Fmar
Ns
Fetch/depth ratio

3

er species. *Forsterygion varium* also had the highest number of canal pores of the four remaining canal systems; however, interspecific differences were less pronounced. The total number of cranial canal pores (all canal pores pooled across the six canal systems) also differed significantly between species (GLM $F_{8, 59} = 60.61$, $p < 0.0001$), and five significant homogeneous groups were detected (Tukey's post hoc tests $p < 0.05$; see table 2 for detailed post hoc test results). *Forsterygion varium* exhibited by far the highest number of canal pores of all species (163 cranial canal pores), and was grouped as significantly different from all remaining species. The second largest number of canal pores was observed in *F. malcolmi* (mean number 118). The remaining species had between 54 and 87 canal pores, a markedly lower number of pores than in the two aforementioned species (table 2). The degree of fetch/depth ratio was significantly correlated with pore number in five of the six canal systems (all regressions had $p < 0.04$; fig. 3), with the exception of the supraorbital canal systems. The linear relationships were positive in all cases (i.e. the number of pores increased with fetch/depth ratio), and the strength of the relationship was similar for the five canal systems (correlation coefficient between 0.25 and 0.40; fig. 3). The linear regression of the total number of cranial canal pores against the wave exposure of the habitat was also significant ($r = 0.34$, $p < 0.004$; fig. 3).

Canal dimensions for the trunk and preopercular-mandibular canals (area at the level of a neuromast, between neuromast positions within a canal, and the canal neuromast area; see fig. 4 for a histological photograph of a canal neuromast) differed significantly between species ($F_{48, 78} = 3.45$, $p < 0.0001$). Follow-up univariate tests showed that, except for the neuromast area of the preopercular-mandibular canal, all other canal measurements showed large and significant interspecific differences ($p < 0.007$ for all five canals; see table 2 for post hoc test results). The most striking pattern that emerged was that all three canal dimension measurements of the preopercular-mandibular canal were approximately 20% larger than those of the trunk canal (table 2). *F. malcolmi* was consistently among the species with the largest cross-sectional canal and neuromast areas for the trunk canal. In contrast, *F. maryannae*, *N. segmentatus* and *F. lapillum* had consistently small cross-sectional canal and neuromast areas. These three species also showed the smallest canal dimensions for the preopercular-mandibular canal. *F. malcolmi* was again among the species with the largest cross-sectional canal and neuromast areas, but *F. capito*, *F. nigripenne*, *F. gymnota* and *F. varium* showed similar large values (for detailed post hoc results, see table 2). Lastly, the neuromast areas of the preopercular-mandibular canal were fairly consistent among species. Overall, the linear regressions showed that there was no relationship between any of the three canal dimensions measured and the degree of fetch/depth ratio ($p > 0.05$ for all measurements).

Neuromasts, cupulae and associated sensory support cells were found to partially occlude the canals, in some cases occupying up to 50% of the canals. Occlusion of the trunk and preopercular-mandibular canals differed significantly between species (GLM $F_{16, 44} = 2.89$, $p < 0.003$); however, univariate tests showed that species differed significantly for the trunk canal ($p < 0.01$), and were only close to significant for the preopercular-mandibular canal ($p = 0.05$). *F. flavonigrum* displayed the least amount

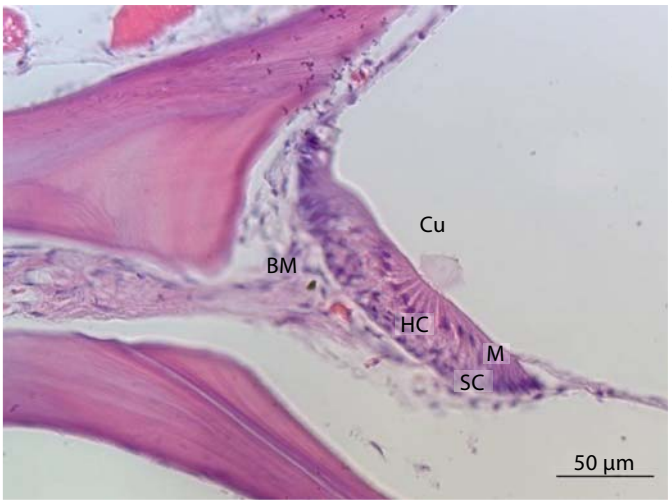


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Fig. 4. Photomicrograph of *F. lapillum* through the preopercular-mandibular canal with a neuromast. Cu = Cuplula; M = mantel cells; HC = hair cells; SC = supporting cells; BM = basal membrane.

Fig. 3. Linear regressions of the mean number of canal pores associated with the five cranial canal systems (see fig. 1), and the total number of cranial canal pores of each species. Data points are represented by the abbreviated species names (species names are abbreviated by the first letter of the genus followed by the first letter of the species name). Dashed lines show 95% confidence intervals. PM denotes the preopercular-mandibular canal. Species are arranged in order of increasing fetch/depth ratio (from left to right; see table 1). The semi-pelagic species *F. maryannae* is highlighted in dark grey and *N. segmentatus* in light grey.

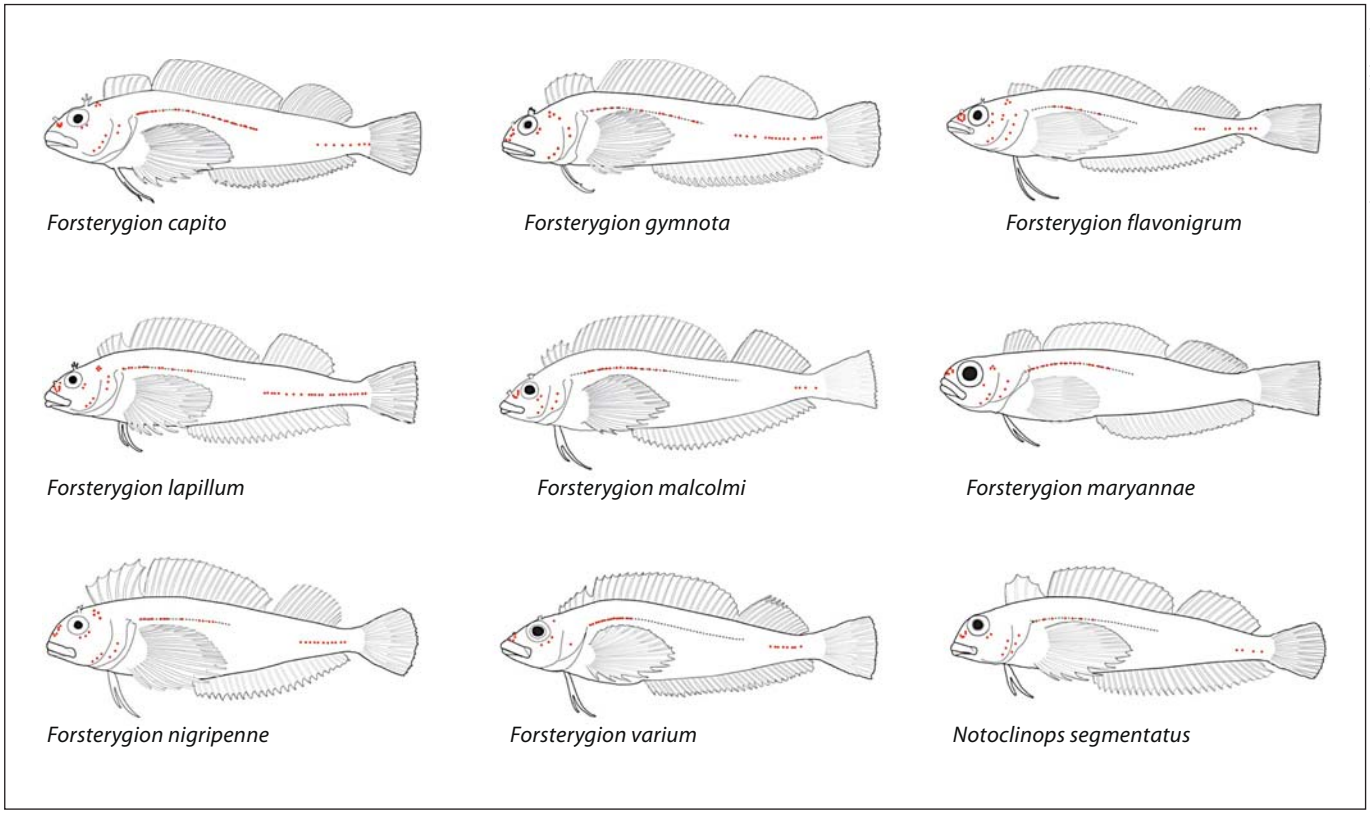

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Fig. 5. Schematic drawings of the distribution and orientation of superficial neuromasts (red dots; black dots in the print version) on the cranial groupings and trunk of all nine triplefin species examined in this study. Note that the canal neuromasts are shown as small grey dots, and that neither the canal nor the superficial neuromasts are drawn to scale.

of occlusion in the trunk canal and differed significantly from the two species with the highest amount of occlusion, *F. gymnota* and *N. segmentatus* (table 2). Fetch/depth ratio did not reveal a significant relationship with the amount of occlusion for either the trunk or the preopercular-mandibular canal ($p > 0.05$ for both canal types).

The distribution and orientation of superficial neuromasts on the head (cranial) and trunk groupings are shown for each species in figure 5. There are seven distinct groupings of superficial neuromasts. Five groups are located on the head (cranial groupings: antorbital, cheek, opercular, postocular and infraorbital grouping), and two groups are located in rows along the trunk. Eight of the nine triplefin species examined displayed a discontinuous lateral line trunk canal (disjunct) and two rows of superficial neuromasts; however, the semi-pelagic species *F. maryannae* had a truncated or foreshortened trunk canal. The majority of superficial neuromasts were associated with canals. There were, however, two exceptions. The first exception was the row of superficial neuromasts associated with the notched scales on the trunk, and the second exception was the antorbital grouping, which is closely associated with the olfactory nares. Again, *F. maryannae* differed from all other species in that it displayed only one row of superficial neuromasts along the trunk. The row of superficial neuromasts present in *F. maryannae* was associated with the trunk grouping. Scales were absent from the areas where superficial neuromasts were found on the head. In contrast, trunk superficial neuromasts were located on notched or pored ctenoid scales (see fig. 6 and 7 for examples of scanning electron photomicrographs of superficial neuromasts), and no neuromasts were observed outside that specific area. No relationship was observed between the number of scales displaying superficial neuromasts and the number of pored scales associated with the trunk. The types of neuromasts located on the head comprised papillate

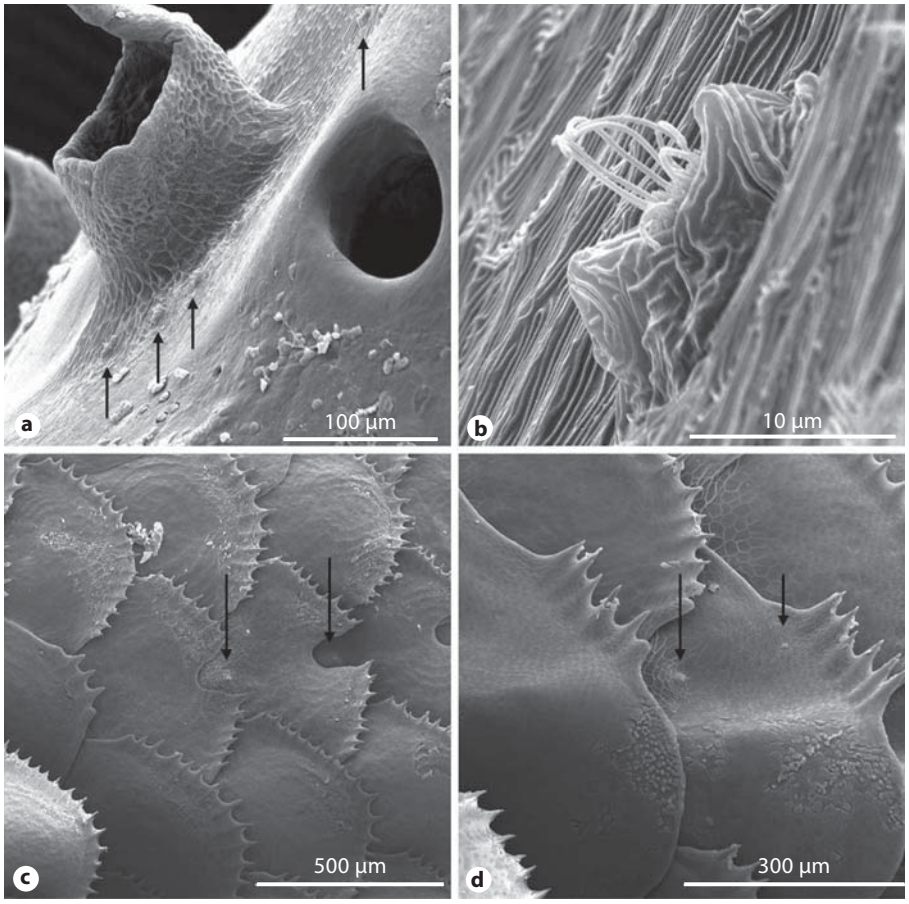

Fig. 6. Scanning electron photomicrographs of *N. segmentatus*. **a** Papillate superficial neuromast from the antorbital region. **b** Close-up of a papillate superficial neuromast from the antorbital region. **c** Flush superficial neuromasts located on notched scales. **d** Flush superficial neuromasts located above the trunk canal.

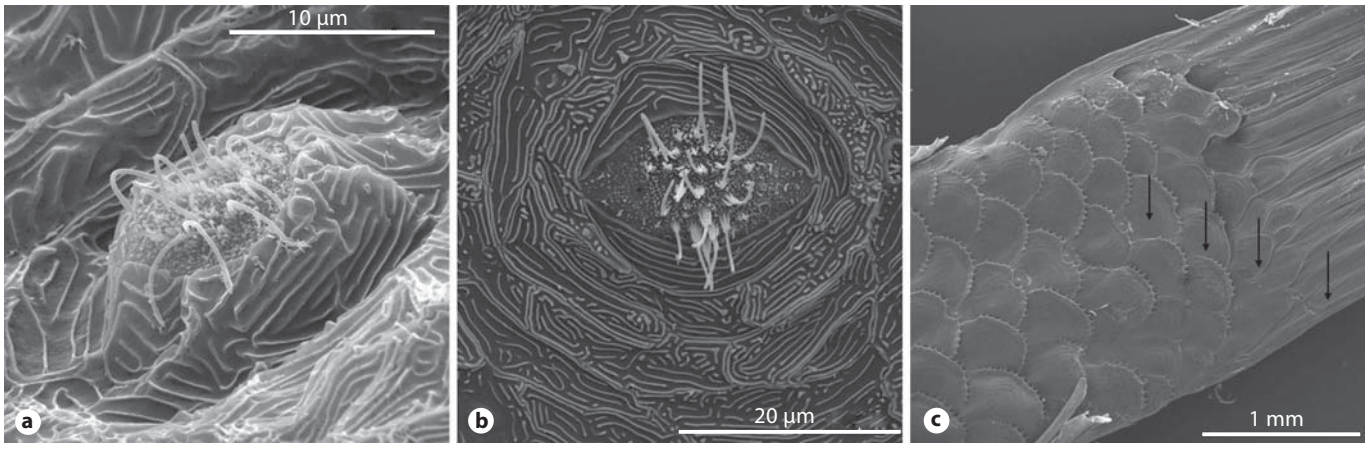

Fig. 7. Scanning electron photomicrographs of *F. gymnota*. **a** Papillate-pit superficial neuromast located near the olfactory nares. **b** Flush superficial neuromast from the trunk region. **c** Superficial neuromasts located on notched scales.

and papillate-pit neuromasts. All species displayed flush neuromasts on the trunk. However, at the caudal end of the body in the ventral row of neuromasts associated with the notched scales, papillate pit-like neuromasts were also observed. Superficial neuromasts were either round or diamond-shaped with hair cells in the middle.

The overall number of superficial neuromasts differed significantly among species (GLM $F_{48,68} = 6.10$, $p < 0.0001$; see table 2 for post hoc test results). Follow-up univariate analyses showed that separate tests on the single groupings were also highly statistically significant for five of the six groupings ($p < 0.005$, for each of the five groupings),

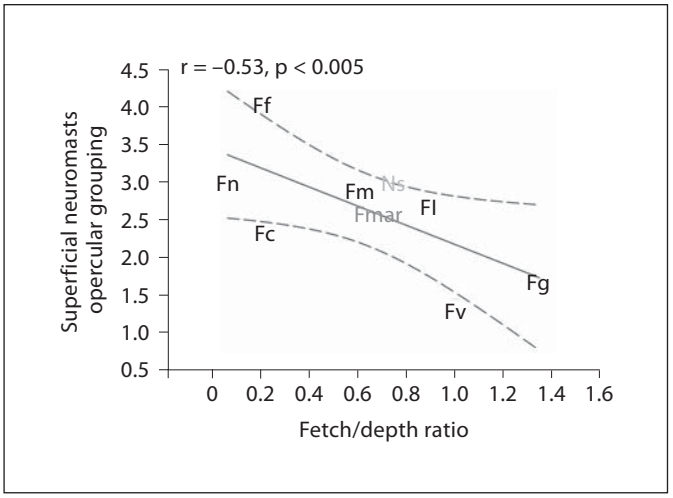

Fig. 8. Linear regression of the number of superficial neuromasts from the opercular grouping against wave energy exposure. Data points are represented by abbreviated species names (species names are abbreviated by the first letter of the genus followed by the first letter of the species name). Dashed lines show the 95% confidence intervals. Species are arranged in order of increasing fetch/depth ratio (from left to right; see table 1). The semi-pelagic species *F. maryannae* is highlighted in dark grey and *N. segmentatus* in light grey.

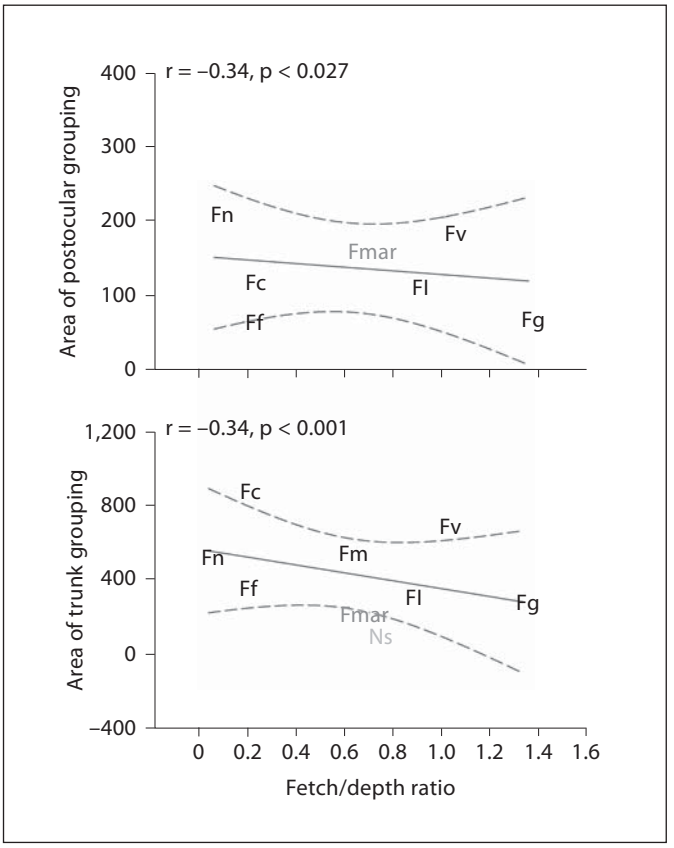

Fig. 9. Linear regressions of the area (μm^2) covered by superficial neuromasts of the postocular grouping and the trunk canal against wave energy exposure. Data points are represented by abbreviated species names (species names are abbreviated by the first letter of the genus followed by the first letter of the species name). Dashed lines show 95% confidence intervals. Species are arranged in order of increasing fetch/depth ratio (from left to right; see table 1). The semi-pelagic species *F. maryannae* is highlighted in dark grey and *N. segmentatus* in light grey.

with the antorbital being the exception ($p > 0.05$). *F. flavonigrum* exhibited the highest number of superficial neuromasts for the infraorbital, opercular, and antorbital groupings, but the lowest for the cheek and among the lowest for the trunk groupings. One species that consistently showed a low number of superficial neuromasts on the groupings was *F. varium,* with a complete absence of superficial neuromast for the cheek grouping (table 2). *Forsterygion malcolmi* had a similarly low number of superficial neuromasts and also a complete absence of any superficial neuromasts for the postocular and cheek groupings (table 2). Both *F. capito* and *N. segmentatus* displayed considerable variation in the number of superficial neuromasts in the different groupings (fig. 7). The opercular grouping displayed a significant, negative relationship with fetch/depth ratio ($r = –0.53$, $p < 0.005$; fig. 8), and two further groupings, the antorbital and the postocular grouping, were close to significant in this respect (antorbital $r = –0.35$, $p = 0.075$; postocular $r = –0.27$, $p = 0.062$).

The superficial neuromast area of the five cranial groupings and the trunk differed significantly among species in all cases (global GLM $F_{8, 98} = 8.80$, $p < 0.0001$, univariate test per species at least $p < 0.005$; table 2). Overall, there was pronounced interspecific variation in the area covered by superficial neuromasts. *F. malcolmi* displayed very large superficial neuromast areas for the opercular, cheek and infraorbital groupings. Similarly, its sister species *F. maryannae* also displayed large neuromast areas for the infraorbital, cheek and postocular groupings. The smallest neuromast area was consistently displayed by *N. segmentatus* (post hoc test always placed this species in a homogenous group with the smallest neuromast areas). *F. lapillum* and *F. gymnota* also displayed low superficial neuromast area. Correlations with fetch/depth ratio were apparent for two groupings, namely the postocular and the trunk grouping. In particular, linear regressions showed that the superficial neuromast area of the postocular ($r = –0.34$, $p = 0.027$; fig. 9) and

trunk ($r = -0.34$, $p < 0.001$; fig. 9) area decreased with fetch/depth ratio. All other groupings showed no significant linear relationship with fetch/depth ratio ($p > 0.05$). Lastly, the density of superficial neuromasts (table 2) showed no significant relationship with fetch/depth ratio.

Discussion

We compared the mechanosensory features of the lateral line systems of all eight *Forsterygion* species and the closely related *N. segmentatus* to investigate the relationship between ecology and lateral line systems. The majority of lateral line systems displayed considerable interspecific variation, and this variation could in some cases be explained by either the wave energy exposure of the habitat that species occupy (e.g. fetch/depth ratio) or by differences in the lifestyle of the species (e.g. pelagic versus benthic lifestyle).

Hydrodynamic Noise as a Selection Pressure in the Evolution of Diverse Lateral Line Morphologies

The numbers of canal pores and superficial neuromasts, and superficial neuromast area, all displayed trends predicted based on the hydrodynamic noise experienced by the species. In particular, the number of canal neuromast pores displayed large interspecific variation, and an increase in the number of canal pores with fetch/depth ratio was observed in five out of the six canal systems, although this relationship was not straightforward among all species. For example, the number of canal pores in *F. gymnota* was lower than predicted given the estimated fetch/depth ratio for this species. This could be explained by the fact that, unlike for other species, the fetch/depth ratio estimate for *F. gymnota* was based on a low sample size (see Materials and Methods), and thus may be inaccurate. Specifically, the sample only included *F. gymnota* that occupied open and exposed reefs, although this species is also found around wharves and jetties [Clements et al., 2000]. For this reason, it seems likely that the wave exposure estimate calculated in our study probably represents an overestimate for this species. Furthermore, *F. malcolmi* had a slightly higher number of canal pores than expected given the wave exposure of the habitat that this species occupies, particularly for the supraorbital, infraorbital, postocular and preopercular-mandibular canal system. The species with the highest canal neuromast number was *F. varium* (total mean 164), which is consistent with the exposed nature of the habitat of this species. Similarly, the six canal systems of the three species that occupy habitats that are the least exposed to high wave energies, *F. nigripenne*, *F. flavonigrum* and *F. capito,* were characterized by a very low number of canal pores (total mean range 62–69). The finding that the canal pore numbers of five out of six canal systems increased significantly with fetch/depth ratio indicates that this ecomorphological trend might be a general feature of the New Zealand triplefin fauna. This result further suggests that the marked habitat diversification [Feary and Clements, 2006; Syms, 1995; Wellenreuther et al., 2007], which appears to be the result of active habitat choice [Wellenreuther and Clements, 2008], may have been associated with the divergence of canal neuromast pores in New Zealand triplefin species. However, factors other than fetch/depth ratio might exhibit an additional effect on lateral line systems in these species. For this reason, we have planned to investigate the relationship between both fish body size and phylogeny on lateral line systems in this clade in a subsequent paper.

In contrast to canal neuromast numbers, superficial neuromast numbers decreased with fetch/depth ratio for several of the groupings. In particular, the preopercular-mandibular canal grouping displayed a statistically significant negative relationship with fetch/depth ratio, and two further canal systems, the antorbital and the postocular system, were close to significant in this respect. Moreover, a similar decline with fetch/depth ratio was observed for the superficial neuromast areas of the postocular and trunk groupings. These findings are again consistent with the predictions based on the habitat use of species, because unlike canal neuromasts, superficial neuromasts are less well suited to function efficiently in environments with strong hydrodynamic forces, as they cannot respond to stimuli in the presence of unidirectional water flow [Engelmann et al., 2000]. Examples of unidirectional water flow in the natural habitats of the species investigated in our study are largely tidally driven, particularly in estuaries and channels (*F. nigripenne* is exclusively found near estuaries, and *F. capito* and *F. lapillum* are also commonly found in shallow areas subject to tidal currents). In addition to that, some of the remaining species in this genus (e.g. *F. varium*) can be found along moderately exposed coastlines [Wellenreuther et al., 2007], and long-period (low-frequency) swells might also produce unidirectional flows.

Role of Paedomorphosis and Pelagic Habitat in the Evolution of Diverse Morphologies

F. maryannae is paedomorphic and has a semi-pelagic lifestyle [Fricke, 1994; Hickey and Clements, 2003]. Fail-

ure of canals to form and replacement of canals with superficial neuromasts have been noted to occur repeatedly in Euteleostei, where they represent a derived paedomorphic state [Coombs et al., 1988; Montgomery et al., 1994; Webb, 1990]. Surprisingly, examination of the distribution and orientation of superficial neuromasts along the trunk area revealed that *F. maryannae* was the only species in the genus *Forsterygion* lacking the second row of superficial neuromasts associated with the notched scales along the trunk canal. We did not measure canal neuromasts along the trunk, and thus we cannot rule out the possibility that trunk canal neuromasts were present. The lack of the second row of superficial neuromasts along the trunk might be linked to the paedomorphic lifestyle of this species. Specifically, *F. maryannae* is commonly found in schools consisting of 10–200 individuals that actively swim at an oblique angle in the water column and feed on zooplankton [Clements, 2003], and both a well-developed canal and superficial neuromast system have been shown to be used in maintaining position in a school [Faucher et al., 2010; Partridge and Pitcher, 1980]. Thus, from a functional viewpoint, the loss of superficial neuromasts along the trunk region could be viewed as a functional hindrance for schooling in this species. It is also noteworthy that *F. maryannae* displayed a relatively large superficial neuromast area for some of the cranial groupings, and this might be related to the unique feeding mode of this species [Feary et al., 2009]. Unlike all other species in this genus, which prey on benthic invertebrates, *F. maryannae* has the ability to capture drifting prey, a behaviour that has been associated with a proliferation of superficial neuromasts [Montgomery and Milton, 1993]. In this sense, the large areas of the superficial neuromasts in the cranial groupings could serve in planktonic prey detection and capture.

Other Factors: Ambient Light Levels, Type of Prey and Predators

The remaining morphological characteristics of the triplefin lateral line systems also displayed considerable interspecific variation; however, this variation could not be explained by differences in fetch/depth ratio, paedomorphosis or pelagic lifestyle. The cross-sectional areas of the trunk and preopercular-mandibular canals varied greatly among species, but showed no relationship with fetch/depth ratio. *F. malcolmi* displayed the largest cross-sectional area (between neuromasts) in the trunk canal, and *F. malcolmi* was also among the species with the largest area for the preopercular-mandibular canal. Three species, *F. maryannae*, *N. segmentatus* and *F. lapillum*, exhibited consistently small areas for both canal types. The lack of a relationship with fetch/depth ratio for these measurements might be related to differences in species-specific agility and activity levels, which affect the amount of self-generated noise that a species experiences. Further research into these factors may provide additional insights into the interspecific differences observed in lateral line systems, particularly those observed for the canal dimensions. *F. nigripenne* was also among the species that possessed narrow cross-sectional areas of the trunk canal (for both between and at the level of neuromasts), which was surprising given that this species is found in very shallow and sheltered habitats (see table 1 and Wellenreuther et al. [2007]). Canals act as low-frequency filters with smaller canals reducing amplitudes at low frequencies [Denton and Gray, 1983, 1988, 1989], thus enhancing the sensitivity towards higher-frequency stimuli [Dijkgraaf, 1962; Gray, 1984]. Wider canals, conversely, typically contain larger neuromasts whose long axis is perpendicular to the canal axis, and hence they are more sensitive to lower frequencies. In a comparative study, Montgomery et al. [1994] showed that a reduction in both the width of canal diameters and neuromast size increases the attenuation of low frequencies, and consequently decreases the overall sensitivity of species to low frequencies. Therefore, the finding that *F. nigripenne* possessed narrow canals suggests the distinct and exclusive estuarine lifestyle of this species [Clements, 2003; Wellenreuther et al., 2009] might have been associated with a reduction of the canal cross-sections. Species inhabiting estuaries regularly experience strong tidal currents, and therefore, substantial amounts of background noise. From this perspective, narrow canals could be of functional importance, as this would help to reduce low-frequency noise which could otherwise mask biologically important signals, such as tidal movements [Denton and Gray, 1988].

Another finding when comparing the canal morphology was that the cross-sectional areas of the preopercular-mandibular canal were on average larger than those of the trunk canal, a pattern that is common in many fish taxa. The neuromasts of the preopercular-mandibular canal were also larger compared to the neuromasts enclosed in the trunk canal, which is consistent with results from other studies [e.g. Hoekstra and Janssen, 1986; Janssen et al., 1987]. Behavioural and physiological studies have demonstrated large neuromasts enhance the sensitivity to lateral line stimuli in the anterior lateral line canals [Hoekstra and Janssen, 1986; Janssen et al., 1992]. Since all benthic triplefin species in this study feed on

small and mobile invertebrates [Feary et al., 2009], enhanced prey sensitivity in the ventral region of the mouth would improve the ability to detect and capture prey.

Low light levels have further been suggested as a selective force in lateral line morphology [Montgomery and Pankhurst, 1997], and a well-developed lateral line system with a proliferation of superficial neuromasts has been related to the ability to capture drifting prey in the absence of visual cues [Montgomery and Milton, 1993]. It is noteworthy that the *Forsterygion* species displaying the greatest number of superficial neuromasts were those inhabiting shallow and sheltered waters, habitats which are often characterised by poor visibility. Specifically, *F. lapillum*, *F. capito* and *F. nigripenne* were among the four species with the highest numbers of superficial neuromasts, and all three species are commonly found in silty, shallow and sheltered habitats [Wellenreuther et al., 2007, 2008]. *F. gymnota* was also among the four species with the highest numbers of superficial neuromasts, although this species is generally found in more exposed habitats [Clements, 2003]. However, the habitat of *F. gymnota* is somewhat unusual as it is associated with sandy or silty shores exposed to wave or tidal action, and which are always turbid due to suspended sediment. The remaining species, which all had lower numbers of superficial neuromasts, are all found in clearer waters [Wellenreuther et al., 2007, 2008]. The relationship between habitat visibility and superficial neuromast number indicates that lateral line systems in the genus *Forsterygion* might have been under selection; however, further work is required to test this hypothesis more explicitly, for example, by quantifying light levels in the habitats and relating this to lateral line morphology.

In conclusion, triplefin species of the genus *Forsterygion* exhibit significant quantitative differences in lateral line systems. Part of this variation can be clearly linked to species-specific differences in lifestyle and fetch/depth ratio. It therefore appears that the morphological divergence in lateral line systems seen in these species is to some degree attributable to the pronounced habitat diversification of this group.

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