Female ornamentation and the fecundity trade-off in a sex-role reversed pipefish

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Abstract

Female ornaments are a rare occurrence in nature. One explanation for this is that female ornaments are costly to produce and maintain and, therefore, females must trade-off resources related to reproduction to promote ornament expression. Here, we investigate the potential trade-off between female ornamentation and fecundity in the sex-role reversed, wide-bodied pipefish, *Stigmatopora nigra*. We measured two components of the female ornament, body width and stripe thickness, and tested the relationship between these ornaments and female fecundity and the mean egg size. Both body width and stripe thickness were strongly and positively related to female body size. After controlling for the influence of body size, we found no evidence of a cost of belly width or stripe thickness on female fecundity. Rather, females that have larger ornaments have higher fecundity and thus a greater relative fitness advantage. However, larger females suffered a slight decrease in egg size, consistent with a potential trade-off between egg size and body size, although no relationship between egg size and ornaments was observed. Our results suggest that larger *S. nigra* females accurately advertise their reproductive value to males, and underscore the importance of investigating the potential mechanisms that promote and maintain honesty of female ornaments.

Introduction

Female ornamentation is a relatively uncommon phenomenon in nature[1,2]. One explanation for its rarity is that female ornamentation comes at a fitness cost in terms of fecundity[3-5]. In general, female investment in reproduction is greater than in males, and female quality is based on her fecundity, the quality of her eggs, and/or parental care investment[2,6]. Female ornaments are therefore not favoured to evolve if their production is costly in terms of future investment into offspring [3]. Yet, despite the potentially high cost to ornament expression, female ornaments occur in diverse taxa, such as insects[7-9], fishes[10,11], reptiles[12,13], and birds[14,15].

Due to a general positive body size-fecundity relationship in many species, larger females may represent a higher reproductive value to prospective mates and perhaps additional ornaments may be unnecessary[9]. However, additional ornaments may serve to amplify information about her quality to choosy males, particularly if ornaments accentuate her body size and, hence, her fecundity[11]. Female ornaments may also be used as a signal in female-female competition where females compete for high quality males that provide direct and indirect genetic benefits to offspring[16,17].

It is hypothesized that sexual signaling imparts a cost in order for the signal to stay honest and thus be maintained in populations[18-21]. Among species where male mate choice is predominant, females are expected to have a further cost in terms of fecundity when it comes to ornament expression[1-3]. If there is no cost, there should be a linear rate of increase in ornament expression and fecundity. Deviations from a positive linear relationship should indicate a trade-off between ornament expression and fecundity. If costs increase with ornament expression, individuals displaying large ornaments would suffer reduced fecundity and demonstrate negative allometry. Alternatively, if costs decrease with ornament expression such

that smaller individuals are saddled with a higher cost, we would expect to see positive allometry.

Members of the family Syngnathidae (pipefish, seahorses, and seadragons) offer an outstanding opportunity to investigate the evolution of female ornamentation because a remarkable diversity of female ornaments has evolved in several lineages, ranging from temporary courtship ornaments to extreme sexual dimorphism, brilliant permanent markings and flashy displays[11,22,23]. Female ornaments ostensibly evolved in syngnathids because of the unique reproductive mode of this group: male pregnancy. Males provide all parental care and species with enclosed brood pouches provide protection, osmoregulation and nutrition to developing embryos[24-28]. In most syngnathid species, male pregnancy decreases the rate at which males can mate but not females, thereby increasing competition between females for access to mating opportunities[29,30]. This discrepancy between reproductive rates between the sexes results in sex-role reversal where sexual selection acts more strongly amongst females than males in many species of syngnathids[31,32]. The expression of female ornamentation in different species appears to be correlated with the strength of sexual selection within and between the sexes, with polyandrous species predicted to have the highest sexual selection in females and therefore displaying the most striking ornaments[11,33].

This study aimed to investigate the trade-off between female ornamentation and fecundity in the wide body pipefish, *Stigmatopora nigra,* Kaup 1856*.* This species is ideal to investigate questions of an ornament-fecundity trade-off because females possess an obvious ornament consisting of a wide belly and the display of a ventral striped ornament to males during courtship. The width of the ornament and stripes can be measured directly and fecundity can be obtained by counting mature ova in the ovaries of females. We explored 1) the relationship between female body size and the size and stripe pattern of the female ornament, 2) the relationship between female fecundity and mean egg size and ornament expression. We predicted that if the ornament is expensive to maintain, more attractive females should produce fewer or smaller eggs to compensate for the cost to female ornament expression.

Material and methods

Study species

The wide body pipefish, *Stigmatopora nigra* (Kaup, 1856), occurs in bays, estuaries and shallow coastal waters of southern Australia and New Zealand[22]. Wide body pipefish breed throughout the year in shallow seagrass beds and their abundance and the proportion of pregnant males reach their peak in September–January[34]. Females possess a wide, laterally compressed body, a darkly pigmented dorsum and a striped, ventral ornament that is displayed to males during courtship (Fig. 1a.). Occasionally, females have an additional fleshy fold on the lateral edges of the ornament[22]. Males do not possess a wide body and lack stripes on their brood pouch (Fig. 1a.). Males have a semi-inverted pouch enclosure and care for offspring until parturition[22,35]. The mating system of the species is unknown due to lack of molecular parentage studies conducted on this species. However, because of the strong sexual dimorphism and possession of a female ornament, the species is most likely polyandrous where males mate with a single female while females can mate with multiple males[33]. This species is also

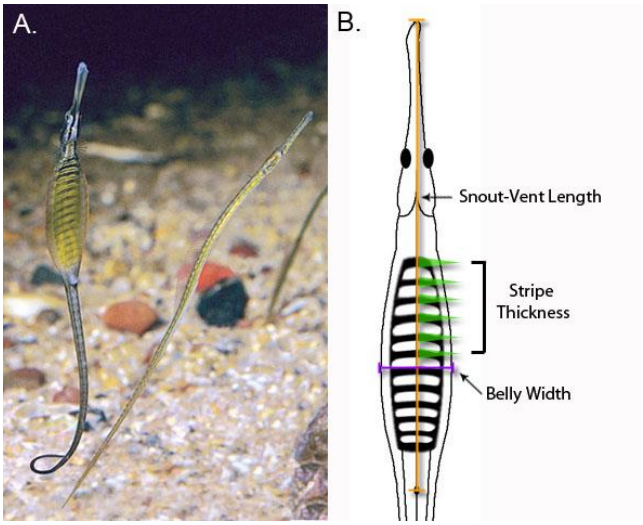

Figure 1. A. Female (left) and male (right) *Stigmatopora nigra*. The female is displaying her striped belly ornament to the male. B. Schematic diagram of measurements used in this study. Snout-vent length is estimated from ventral photographs from the tip of the rostrum to the anal pore. Stripe thickness is the mean width of the first six dark stripes. Belly width is calculated as the widest part of the body. Photography © Rudie Kuiter, used with permission.

putatively sex-role reversed with respect to sexual selection (i.e., sexual selection acting more strongly on females than males) similar to other pipefish species that display female ornaments[32,36].

Sample collections

Adult *S. nigra* used in the study were museum specimens collected either by using drop or seine nets at Grassy Point in Port Phillip Bay on the Bellarine Peninsula, Victoria, Australia (38°07′ S, 144°41′ E). Specimens were sampled across multiple years (1997, 1999, 2005, and 2006) during September – January as part of a series of unrelated studies[37-39]. These fish were euthanized immediately after capture using either a 99% ethanol solution or benzocaine before being preserved in 70% ethanol and stored in the collection at Museum Victoria.

Morphological measurements

Female pipefishes were photographed using a Nikon D80 digital SLR camera for morphological measurements. Females were placed on a foam board covered by a sheet of laminated paper with 1mm grid lines for scale. The females were then pinned down flat with their ventral side exposed, as this allowed for accurate measures of both body size and ornamental traits. Measurements were then taken from the photographs using the image analysis software ImageJ (http://rsb.info.nih.gov/ij/). Because 19% of females and 24% of males had broken tails, we used snout-vent length (SVL, tip of rostrum to anal pore, Fig. 1b) as a measure of body size as opposed to total length (TL, tip of rostrum to tip of tail). Snout-vent length was highly correlated to total length in both sexes (female $F_{1,102} = 835.1$, $p<0.001$; male $F_{1,54} = 395.2$, $p<0.001$). We obtained two different measures of female ornamentation: belly width and stripe thickness. Belly width was measured from the widest point perpendicular to SVL to ensure uniformity of measurements (Fig. 1b). We also measured belly width in males. For stripe thickness, the width of dark stripes at the midpoint of the SVL axis was measured (Fig. 1b). While preservation caused fading of the dark stripes on many females, the first six stripes were visible for the majority of the females. Therefore, the mean thickness of the first six stripes was used as the measurement for stripe thickness.

Dissections and egg size measures

After photography, females were placed in a petri dish and submerged in water to prevent desiccation. Ovaries were dissected from females and eggs gently separated from ovarian tissue using tweezers, enumerated to estimate fecundity, and the egg diameters measured under 40X magnification using a 0.1mm graticule. Due to the ovoid shape of most eggs, two perpendicular measures of diameter were taken and averaged in order to estimate mean egg size.

Female ovaries contained both immature and mature (hydrated) eggs, although preservation made it difficult to identify the two. Counting immature eggs would overestimate a female's current fecundity, or potential clutch size, and thus eggs available to a male during mating. Therefore, we developed a method to estimate which eggs were mature in female ovaries, and hence her fecundity using the size range of newly laid eggs (without eyespots) located within the brood pouch of ethanol-preserved males. First, eggs from each male's brood pouch were dissected, enumerated for an estimate of male reproductive success and the diameters measured under 40X magnification using a 0.1mm graticule. Second, linear regression was used to find the relationship, across males, between the minimum and maximum egg sizes in a pouch. A significant positive relationship was found between the minimum and maximum egg size found in a male's brood pouch ($F_{1,57} = 20.9$, $p<0.001$). The maximum egg diameter was found to correlate to 0.613*the minimum egg diameter found in a male's pouch + 0.587. Third, assuming that each male has eggs from just one female in his pouch (because the range of male egg numbers is contained within the range of female fecundities, Table 1), we used this equation to predict the smallest mature egg size of a female, dependent on the largest egg size found in her ovary. All eggs that fell within this size range were counted as mature for that particular female.

Statistical analysis

We analysed data from 104 females and 59 males for which all metrics were available (SVL, fecundity, egg size, belly width and stripe thickness in females; egg number and egg size in males,

Table 1. The number (n) of male and female *Stigmatopora nigra*, mean total length (TL), mean snout-vent length (SVL), mean fecundity (number of eggs), mean egg size (egg size), and mean belly width (belly width) and mean stripe thickness (stripe thickness) of females. All means are reported ± one standard error of the mean. *na* = not applicable.

	n	TL (cm)	SVL (cm)	Fecundity	Egg size (mm)	Belly Width (mm)	Stripe Thickness (mm)
Female	104	9.73 ± 0.12	4.47 ± 0.06	32.9 ± 1.4	0.92 ± 0.01	4.53 ± 0.10	0.76 ± 0.01
Male	59	8.26 ± 0.18	3.61 ± 0.08	32.7 ± 1.9	0.95 ± 0.01	2.15 ± 0.05	*na*

Table 1.). Larger females had proportionately larger ornaments (SVL vs. belly width: $F_{1,102}$ = 215.8, p<0.001; SVL vs. mean stripe thickness: $F_{1,102}$ = 146.7, p<0.001). Therefore, before further analysis was performed, ornament traits were standardized for female body sizes by using the raw residuals from ordinary least squares regressions of ornament ~ SVL.

To investigate the relationship between female ornaments and fitness correlates, we modelled female fecundity and egg size as a function of female size-adjusted ornamentation and SVL using linear mixed effects models fitted in the lme4 package in R 3.3.1[40]. We included a random intercept for sample year to account for potential among-year differences in reproductive investment driven by unmeasured environmental conditions. We compared a series of increasingly complex models (fitted with maximum likelihood) that included linear and quadratic terms for ornamentation and SVL. We used Akaike Information Criterion corrected for small sample size AICc, [41] to select the best fit model. Fecundity data was natural log-transformed to satisfy model assumptions and predictor variables centred to facilitate interpretation of polynomial terms. Parameter estimates and 95% credible intervals were derived from the posterior distribution of the fixed effects in the best models (fitted with restricted maximum likelihood, REML) using 1000 model simulations generated by the arm R package.

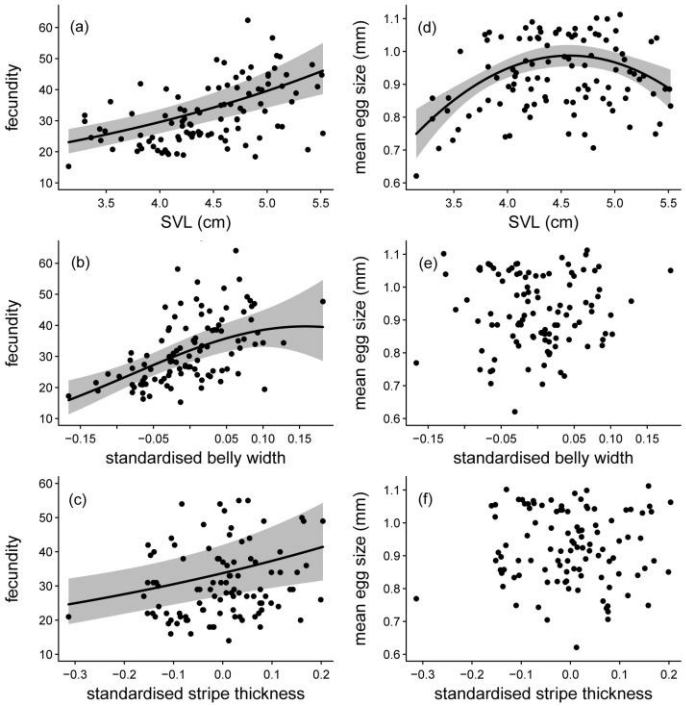

Figure 2. Predicted relationship (grey areas are ± 95% CI) between (a) fecundity and snout-vent length (SVL); (b) fecundity and standardised belly width (see methods); (c) fecundity and standardised mean stripe thickness (see methods); (d) mean egg size and SVL; (e) mean egg size and standardised belly width; (f) mean egg size and standardised stripe thickness. Points in (a) and (b) represent partial effects from multiple mixed model regression (other covariates are held at mean values). Points in c-f are observations.

Results

Sexual dimorphism is apparent in this species: females were larger and have bellies that were twice the width of males, on average (ANOVA SVL: $F_{1,161}$ = 82.6, p <0.0001; ANOVA belly width: $F_{1,161}$ = 440.8, P <0.0001; Table 1). Females varied widely in belly width (2.2-6.7mm), stripe thickness (0.38-1.1mm) and fecundity (14-89 eggs). Male brood pouches contained between 1 and 76 eggs. Fecundity was curve-linearly related to mean egg size ($\beta_{(egg)}$= 1.132 [0.626 to 1.650 95%CI], $\beta_{(egg^2)}$= -6.459 [-10.492 to -2.459 95%CI]), with females of intermediate fecundity having the largest eggs. The best fecundity model predicted by morphological traits included linear and quadratic terms for standardised belly width and a linear term for SVL. Larger females were more fecund ($\beta_{(SVL)}$= 0.297 [0.195 to 0.395 95%CI], Fig. 2a). Females

Table 2. Results of AICc based model selection for female fecundity and mean egg size. SVL=snout-vent length, stand.BW=standardised belly width; stand.ST=standardised stripe thickness; k=number of model parameters. The best model for each reproductive measure (ΔAICc= 0) is highlighted in bold.

Model	k	Fecundity ΔAICc	Egg size ΔAICc
null	3	41.9	11.3
SVL	4	26.9	8.2
SVL + SVL^2	5	29.0	**0.0**
stand. BW	4	26.8	12.6
stand. BW + stand. BW^2	5	28.5	13.0
stand. BW + SVL	5	0.7	8.9
stand. BW + stand. BW^2 + SVL	6	**0.0**	10.2
stand. BW + stand. BW^2 + SVL + SVL^2	7	5.3	3.3
stand. ST	4	34.1	13.3
stand. ST + stand. ST^2	5	35.8	15.5
stand. ST + SVL	5	16.6	10.3
stand. ST + stand. ST^2 + SVL	6	18.5	11.9
stand. ST + stand. ST^2 + SVL + SVL^2	7	20.3	3.6

with relatively larger belly widths also had higher fecundity ($\beta_{(stand.BW)}$= 2.602 [1.703 to 3.617 95%CI], $\beta_{(stand.BW^2)}$= -8.518 [-18.266 to 1.158 95%CI], Fig. 2b). The best egg size model included linear and quadratic terms for SVL (Fig. 2d). Medium sized females had the largest eggs ($\beta_{(SVL)}$= 1.066 [0.597 to 1.498 95%CI], $\beta_{(SVL^2)}$= -0.116 [-0.165 to -0.063 95%CI], Fig. 2c). There was no relationship between standardized stripe thickness and fecundity or egg size (Fig. 2e, 2f).

Discussion

We found strong evidence for a positive relationship between ornament expression and fecundity in *S. nigra*, and that the two traits related to the female ornament of *S. nigra* are highly correlated to body size. Together, these results emphasise that bearing ornaments does not have to come at a cost to fecundity. However, females with proportionately larger ornaments may trade-off egg size with ornament expression. Such a trade-off may allow larger females to produce more eggs than smaller females, although this might incur a potential cost to offspring fitness e.g. [42,43]. We argue that benefits associated with the production and maintenance of female ornaments in *S. nigra* outweigh any direct costs to fecundity, but perhaps not quality of offspring if egg size is related to offspring fitness.

The two components of the female ornament in *S. nigra*, body width and stripe thickness, scaled with body size and had a positive relationship with fecundity. Ornaments often scale by traits such as body mass and/or size and are frequently condition dependent[44-47]. In such cases, larger or better condition females should have proportionately larger ornaments. In *S. nigra*, body size is an accurate indicator of ornament expression and fecundity, suggesting that body size alone would honestly signal the reproductive status of females and potentially female quality. This would then set the stage for mate choice by males for body size to be maintained via direct benefits[48], particularly if females are competing over mating opportunities or male quality[17,49].

We expected to find a cost for having an elaborate ornament in *S. nigra* that would negatively impact fecundity in the most attractive females (i.e., females with largest ornaments) because the exaggerated width of the belly in females is potentially energetically expensive to maintain and

may be a handicap in terms of limiting movement and predator escape[11,50]. In the case of *S. nigra*, fecundity demonstrates positive allometry with the size of the ornament such that females with larger ornaments have higher fitness gains over females that have smaller ornaments. Positive allometry between female ornamentation and fecundity are demonstrated in diverse taxa. For example horned beetle, *Onthophagus sagittarius*[47], dance flies, *Rhamphomyia tarsata*[5], blue throats, *Luscinia svecica*[45], and glow worms, *Lampyris noctiluca*[9], all show a positive relationship of ornaments to body size. One potential explanation for positive allometry in this and other species is that smaller individuals may suffer a higher cost to fecundity than larger individuals. This may then set the stage for a trade-off between reproduction and growth [51,52].

Several studies have shown correlations between female ornaments and phenotypic quality of individuals or offspring[53,] but see [54]. In a recent study it was shown that non-mobile glow worm females attract mobile males by emitting a glow that is an honest signal of the female's fecundity[9]. Among pipefish, female ornaments are often condition-dependent and can honestly signal reproductive potential[55], or be preferred by males during courtship[56]. Moreover, Cuhna and colleagues[57] found female black striped pipefish, *Syngnathus abaster*, possessing darker stripes have larger offspring at birth, lending support that female ornaments in pipefish accurately reflect a female's reproductive potential.

In this study, mean egg size showed a positive curve-linear relationship with body size and fecundity, a common theme for many species, including pipefish[58-60]. Egg size is often related to offspring fitness, with larger eggs bestowing higher fitness benefits to developing young[61,62]. Among pipefish, egg size is generally related to offspring fitness[63] although females may strategically distribute resources to eggs and clutches depending on mate quality[42,64].

In our study, mean egg size was not related to stripe thickness, yet the smallest and largest females had proportionately smaller eggs suggesting the potential for a cost to producing larger eggs. One potential explanation is that female *S. nigra* that breed more may have fewer resources to provision future broods and egg size may decrease as a result of resource depletion[65]. However it is currently unknown if *S. nigra* are capital breeders (i.e., pay for reproduction based on stored resources, *sensu*[66]) or if larger females are more successful at breeding. Alternatively, larger females may strategically decrease their egg size to increase fecundity[60,67]. Reduction in egg and/or clutch size is hypothesized to be adaptive in in species if the cost of reproduction declines with increasing age and age-selective mortality is low relative to reproduction-dependent mortality [68]. However, it is unclear whether larger eggs have higher fitness than smaller eggs and this assumption would need to be established before invoking an adaptive explanation. Finally, one issue to bear in mind is that all females were collected in the wild and may have recently mated affecting the fecundity and potentially the egg size of particular females. Currently, the ovarian type in *S. nigra* is unknown, although at least two types have thus far been described in pipefishes: asynchronous type where females may produce small numbers of eggs continuously such as in *Syngnathus scovelli* and *Syngnathus typhle*[69], or group-synchronous type such as in *Nerophis ophidion*[70] and *Corythoichthys haematopterus*[71] where ovaries are mature in distinct clutches. Because mature egg size was deduced on the relative size of eggs within the ovaries based on the size of eggs within males, it is possible that we overestimated fecundity by including some non-mature eggs. Thus, if larger females recently mated and have a high proportion of non-mature eggs in the ovaries, this may account for the reduced mean egg size in the largest females.

One relatively unexplored aspect to the evolution of female ornamentation is their use in intra-sexual competition[17,72]. Currently, little is known if the ornament in *S. nigra* is used primarily to signal males during courtship or in competitive interactions between females by supressing display times of rival females. Future studies should quantify the types of interactions between females and potential mates versus potential rivals to help elucidate the nature of the female ornament in this species.

To conclude, in contrast to what is known about male sexual ornaments, far less is known about the causes and consequences of the evolution of female ornamentation. Here, we found no evidence of a fecundity cost associated with the expression of an extravagant female ornament in

a sex role-reversed pipefish, although a potential trade-off between egg size and ornament size was uncovered. Future studies should investigate whether female competition, male mate choice, or a combination of the two are important to the evolution and maintenance of female ornaments in this and other species in nature.

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Author contributions

MW and BBMW initiated the study. MW performed all dissections with the assistance of DB. KBM, JRM and MW analysed the data. KBM drafted manuscript with help from JRM, MW, DB, BBMW. All authors gave final approval for publication.

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