

Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish

John R. Morrongiello^{1,2,3*}, Nicholas R. Bond^{1,2,4}, David A. Crook^{2,5,6} and Bob B. M. Wong¹

¹School of Biological Sciences, Monash University, Clayton, Vic 3800, Australia; ²eWater Cooperative Research Centre, Canberra, ACT 2600, Australia; ³CSIRO Wealth from Oceans Flagship, Hobart, Tas 7001, Australia; ⁴The Australian Rivers Institute, Griffith University, Nathan, QLD 4111, Australia; ⁵Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, Heidelberg, Vic 3084, Australia; and ⁶Research Institute for Environment and Livelihoods, Charles Darwin University, Darwin, NT 0909, Australia

Summary

1. Maternal reproductive investment is thought to reflect a trade-off between offspring size and fecundity, and models generally predict that mothers inhabiting adverse environments will produce fewer, larger offspring. More recently, the importance of environmental unpredictability in influencing maternal investment has been considered, with some models predicting that mothers should adopt a diversified bet-hedging strategy whilst others a conservative bet-hedging strategy.

2. We explore spatial egg size and fecundity patterns in the freshwater fish southern pygmy perch (*Nannoperca australis*) that inhabits a diversity of streams along gradients of environmental quality, variability and predictability.

3. Contrary to some predictions, *N. australis* populations inhabiting increasingly harsh streams produced more numerous and smaller eggs. Furthermore, within-female egg size variability increased as environments became more unpredictable.

4. We argue that in harsh environments or those prone to physical disturbance, sources of mortality are size independent with offspring size having only a minor influence on offspring fitness. Instead, maternal fitness is maximized by producing many small eggs, increasing the likelihood that some offspring will disperse to permanent water. We also provide empirical support for diversified bet-hedging as an adaptive strategy when future environmental quality is uncertain and suggest egg size may be a more appropriate fitness measure in stable environments characterized by size-dependent fitness. These results likely reflect spatial patterns of adaptive plasticity and bet-hedging in response to both predictable and unpredictable environmental variance and highlight the importance of considering both trait averages and variance.

5. Reproductive life-history traits can vary predictably along environmental gradients. Human activity, such as the hydrological modification of natural flow regimes, alters the form and magnitude of these gradients, and this can have both ecological and evolutionary implications for biota adapted to now non-existent natural environmental heterogeneity.

Key-words: adaptive phenotypic plasticity, environmental quality, freshwater fish, maternal effect, optimal offspring size, reproductive life history, spatial bet-hedging, unpredictable environment

Introduction

The partitioning of a mother's resources among her offspring has received considerable theoretical and empirical attention (Bernardo 1996; Fox & Czesak 2000). Often viewed as a trade-off given finite resources, mothers can either produce many small or a few large offspring, with the outcome being a balance between the benefits of increased fecundity or

higher quality, larger progeny. Many life-history models (e.g. Smith & Fretwell 1974; Brockelman 1975; McGinley, Temme & Geber 1987; Einum & Fleming 2004) predict that a single optimal offspring size should be produced, conditional on the environmental cues experienced by a mother. All else being equal, tactical variation in offspring size and number among populations should mirror differences in these optima, with offspring size declining and number increasing with increasing environmental quality or decreasing environmental variability. Such patterns have been observed across

*Correspondence author. E-mail: john.morrongiello@csiro.au

taxa including plants (Vaughton & Ramsey 1998), insects (Zovi *et al.* 2008), marine invertebrates (Marshall & Keough 2008), amphibians (Rasanen *et al.* 2008), birds (Tremblay *et al.* 2003) and fish (Johnston & Leggett 2002).

In their basic form, optimality models make two important assumptions: (i) larger offspring are fitter, and (ii) environmental conditions are predictable. Empirical data generally, but not always, support the first assumption (Einum & Fleming 1999, 2000; Moran & Emler 2001; but see Dibattista *et al.* 2007; Allen, Buckley & Marshall 2008). In reality, the second assumption is often a simplification, and more recent offspring size models have allowed environmental quality to vary (McGinley, Temme & Geber 1987; Lalonde 1991; Einum & Fleming 2004). It is also important to note that whilst variable environments are relatively unpredictable in many circumstances (Poff & Ward 1989; Kennard *et al.* 2010), this is not always the case (Colwell 1974), so it is valuable to investigate the concurrent effects of both environmental variability and predictability on maternal reproductive investment strategies and tactics (Fischer, Taborsky & Kokko 2011).

Despite predictions of optimality, considerable phenotypic variation in offspring size and number has been observed empirically on a number of scales, including variation among females within a population, among a female's clutches through time as well as within a single clutch (Bernardo 1996; Kaplan & King 1997; Kudo 2001; Lips 2001; Johnston & Leggett 2002; Marshall & Keough 2008). Classic optimality models therefore do not adequately explain departures from canalized optimal trait values. Often, this additional variation in offspring size has been attributed to the physiological inability of females to produce offspring of uniform size (constraining effects: Fox & Czesak 2000; Einum & Fleming 2004), a consequence of mothers producing small clutch sizes (Ebert 1994; Charnov & Downhower 1995), a weakening of selection in variable environments (McGinley, Temme & Geber 1987) or differences in maternal phenotype (body size and fecundity effects: Parker & Begon 1986; reviewed by Marshall *et al.* 2010). More recently, within-female variation has been considered an adaptive response to environmental unpredictability (Koops, Hutchings & Adams 2003; Marshall, Bonduriansky & Bussiere 2008; Crean & Marshall 2009).

Individuals inhabiting unpredictable environments are exposed to stochastic selection pressures that can strongly influence the evolution and expression of life histories (Stearns 1992). When environments possess reliable cues as to their future state, mothers can display phenotypic plasticity and adjust offspring sizes accordingly (e.g. seed beetles *Staler limbatus*; Fox, Thakar & Mousseau 1997). However, when the future status of environments is unpredictable, bet-hedging strategies may be favoured that, whilst having reduced arithmetic fitness in the current generation, have an evolutionary advantage through reduced fitness variance across generations (Seger & Brockmann 1987). In other words, it is better to have some success in each reproductive event rather than having a lot of success in one event and none in the next: for example, by producing a range of off-

spring phenotypes so that at least some will match local environmental conditions (Crean & Marshall 2009). More general life-history models have long predicted the benefits of producing offspring with variable phenotypes in unpredictable environments (e.g. Cohen 1966), but this has not been reflected in theory specific to offspring size (Marshall, Bonduriansky & Bussiere 2008).

Bet-hedging strategies lie along a continuum, ranging from situations where mothers produce a range of offspring sizes to spread the risk of incorrectly predicting the future and ensuring at least some offspring survive (diversified bet-hedging), to situations where mothers are risk averse (conservative bet-hedging) and produce offspring larger than those predicted to be optimum in a stable environment with the same long-term mean quality (Einum & Fleming 2004; Simons 2011). Bet-hedging theory has been further expanded to include situations where a variety of offspring phenotypic characteristics (e.g. seed germination time), not just size, can be favoured in unpredictable environments (reviewed by Childs, Metcalf & Rees 2010). Whilst adaptive within-female offspring variation is beginning to receive both theoretical (Marshall, Bonduriansky & Bussiere 2008; but see McGinley, Temme & Geber 1987; Einum & Fleming 2004) and empirical support (Koops, Hutchings & Adams 2003; Marshall, Bonduriansky & Bussiere 2008; Crean & Marshall 2009; Simons 2011), further work is needed to explore the relative importance and causal mechanisms of average offspring size and offspring size variability on a range of spatial and temporal scales. It seems likely that both adaptive plasticity and bet-hedging in offspring size will co-evolve when both predictable and unpredictable environmental variances are present and influence fitness (reviewed by Simons 2011).

The quality, variability and predictability of environments experienced by freshwater biota are primarily driven by differences in hydrological regime (Poff *et al.* 1997; Lake 2000; Naiman *et al.* 2008). Hydrology directly affects a range of processes such as physical disturbance, habitat connectivity and persistence, resource availability and the form and intensity of biological interactions (Schlosser 1987; Jackson, Peres-Neto & Olden 2001; Lake 2003). In turn, the spatial and temporal extent of these abiotic and biotic responses to the hydrological regime can have direct implications for the evolution of life histories and phenotypic expression in aquatic biota (Wellborn, Skelly & Werner 1996; Reznick, Butler & Rodd 2001; Johnston & Leggett 2002; Lytle & Poff 2004; Arendt & Reznick 2005).

Egg size can vary among populations, among females within a population and within-individual females. Here, we document the relative importance of each of these levels of egg size variation in a freshwater fish, the southern pygmy perch (*Nannoperca australis*), and explore the potential for egg size variability to be an adaptive response to predictable and unpredictable environmental variance. First, we explore among-population variation in average egg size and number along gradients of environmental quality and temporal variability to test the optimality model predictions that average egg size will increase and egg number will decrease as

environmental quality declines and environmental variability increases (Smith & Fretwell 1974; McGinley, Temme & Geber 1987; Einum & Fleming 2004). Second, we investigate within-female and among-female egg size variation along a gradient of environmental predictability to test whether egg size variability itself is reflective of an adaptive bet-hedging strategy. A positive relationship between within-female or among-female egg size variation and environmental unpredictability, coupled with a decrease in average egg size, is evidence for diversified bet-hedging (Kaplan & Cooper 1984; Koops, Hutchings & Adams 2003; Marshall, Bonduriansky & Bussiere 2008), whereas the opposite is indicative of conservative bet-hedging (Einum & Fleming 2004). Finally, we synthesize the among-population, among-female and within-female results to explore the potential for predictable and unpredictable environmental variation to concurrently influence fitness.

Materials and methods

STUDY SPECIES

The southern pygmy perch, *Nannoperca australis* Günther, is a small bodied (<90 mm) freshwater fish found throughout south-east Australia and inhabits a broad range of environments, ranging from large rivers and perennial streams to wetlands and ephemeral creeks. *Nannoperca australis* naturally occurs along gradients of environmental quality, variability and predictability with drought being a particularly important form of physical disturbance for fish in these systems (Bond & Lake 2005; Bond, Lake & Arthington 2008). The suite of piscivorous species to which *N. australis* is exposed generally increases with stream size (Lake 1982; J.R. Morrongiello pers. obs.), suggesting agreement with the widely held pattern of diminishing

biotic-factor importance in shaping aquatic communities as streams become more ephemeral (Schlosser 1987, 1990).

Female *N. australis* sexually mature in their first year and few survive to breed in a second season (Humphries 1995). They are protracted spawners (Llewellyn 1974) and release non-adhesive eggs onto the substrate within-male territories (Mitchell 1976), possibly indicating weak paternal protection of eggs and larvae (Knight *et al.* 2007). Importantly, previous studies have shown that *N. australis* egg size and fecundity can vary considerably among populations (Llewellyn 1974; Humphries 1995; Woodward 2005), and it has been suggested that this variation is reflective of life-history trade-offs in response to density-independent factors such as hydrological variation and physiochemical stress (Humphries 1995; Morrongiello *et al.* 2011). *Nannoperca australis* develop in the egg for approximately 3 days and larvae hatch at ~3.5 mm in length (Llewellyn 1974). These protolarvae are not well developed and are initially poor swimmers that remain on the bottom for long periods (J.R. Morrongiello pers. obs.). Protolarvae are wholly dependent on their yolk sac until exogenous feeding begins ~5 days post-hatching (Llewellyn 1974). Whilst the relationship between egg size and offspring performance is not known in *N. australis*, larger larvae have greater survival under abiotic stress and swim faster (Morrongiello 2011), possibly reflecting a positive asymptotic performance function where larger eggs produce fitter offspring as seen in other fishes (e.g. Einum & Fleming 1999, 2000). However, the specific form (e.g. asymptotic or peaked) and parameterization (e.g. asymptote or peak position, skewness, symmetrical or asymmetrical) of the offspring size: performance function are likely to vary considerably through space and time (e.g. Hendry, Day & Cooper 2001; Dibattista *et al.* 2007; Allen, Buckley & Marshall 2008; Marshall & Keough 2008).

STUDY SITES AND SAMPLE COLLECTION

Sexually mature female *N. australis* ($n = 312$) were collected from 10 streams, on 20 occasions across 3 years (Fig. 1, Table 1), using a

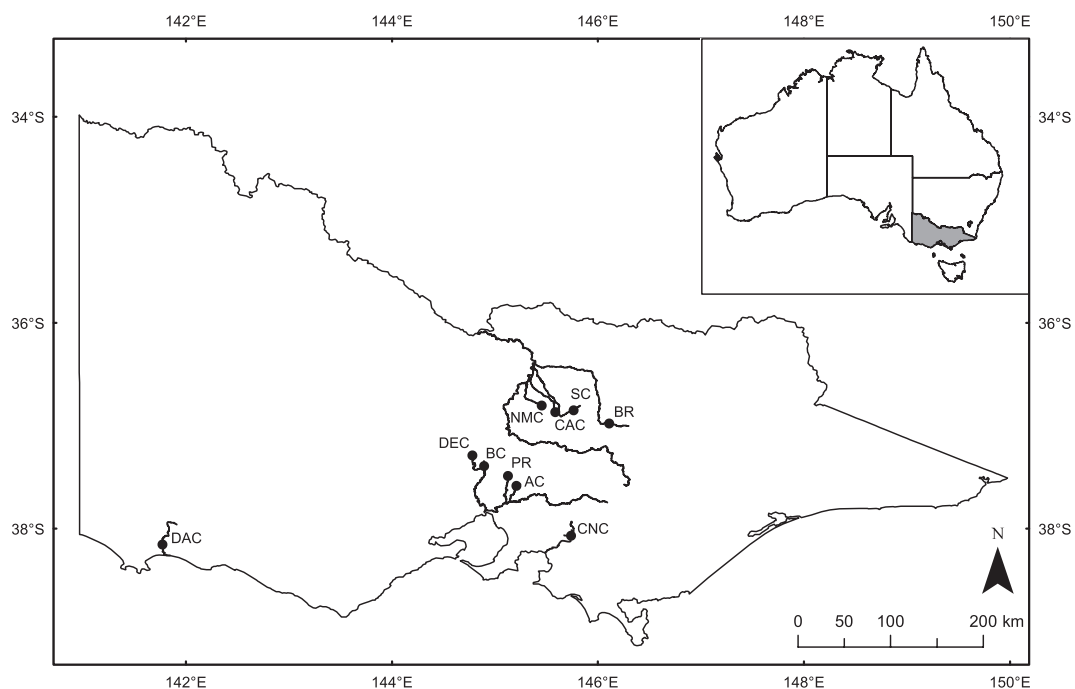


Fig. 1. Location of study sites in Victoria, Australia. AC: Arthurs Creek; BC: Boyd Creek; BR: Broken River; CNC: Cannibal Creek; CAC: Castle Creek; DAC: Darlots Creek; DEC: Deep Creek, NMC: Nine Mile Creek; PR: Plenty River; SC: Seven Creeks.

Table 1. Description of samples used in this study

Stream name	Longitude	Latitude	Date	Day length (h)	<i>n</i>	Hydrological data	
						Source	Time series length (years)
Arthurs Creek	37°34'55-0"S	37°34'55-0"E	15 Aug 2007	10-65	5-8	G	29
Boyd Creek	37°23'19-2"S	37°23'19-2"E	26 Sept 2007	12-23	18-20	G	30
			30 Oct 2007	13-57	20	G	30
Broken River	36°58'33-2"S	36°58'33-2"E	29 Aug 2007	11-17	11-17	M	> 15
			9 Nov 2007	13-88	12-18	M	> 15
			21 Aug 2008	10-92	12	M	> 15
Cannibal Creek	38°3'57-0"S	38°3'57-0"E	24 Sept 2007	12-17	8-9	G	18
			8 Oct 2007	12-73	7	G	18
Castle Creek	36°51'53-2"S	36°51'53-2"E	13 Aug 2007	10-63	19-20	M	> 15
			22 Sept 2007	12-10	19-26	M	> 15
			20 Aug 2008	10-9	14-15	M	> 15
			22 Jul 2009	10-02	10-12	M	> 15
Darlots Creek	38°9'1-0"S	38°9'1-0"E	16 Oct 2007	13-07	13	G	38
Deep Creek	37°17'9-0"S	37°17'9-0"E	26 Sept 2007	12-25	15-20	G	30
			30 Oct 2007	13-57	15-16	G	30
Nine Mile Creek	36°47'59-0"S	36°47'59-0"E	20 Aug 2008	10-83	8-9	M	> 15
Plenty River	37°29'10-6"S	37°29'10-6"E	15 Aug 2007	10-65	3	M	> 15
			9 Oct 2007	12-77	16-27	M	> 15
Seven Creeks	36°50'47-9"S	36°50'47-9"E	22 Sept 2007	12-08	15-29	G	42
			23 Oct 2008	13-3	9-11	G	43

n sample size range (min. to max. across trait measurements); source: G stream gauge, M modelled flow data where no gauge data available. Modelled flow extrapolated using catchment characteristics from 120 gauged streams in Victoria, Australia with a minimum of 15 years data (Bond *et al.* 2011).

combination of fyke nets and bait traps. Sexual maturity was determined by the ability to easily express clear eggs from females when gently squeezed (no more than 5–10 fish⁻¹). Fish were killed by administering an overdose of clove oil. Total length (*length*) was measured to the nearest mm. Fresh, mature eggs were expressed from mothers to provide a point estimate of egg size and allowed to water harden for approximately 10 min before being photographed with a Nikon D80 camera (Nikon Inc., Tokyo Japan) fitted with a macro lens. Fish were then preserved in 95% ethanol.

TRAIT MEASUREMENTS

The cross-sectional area of eggs (average 37 fish⁻¹, ~8.7% of fecundity) was measured from digital photographs using IMAGE J version 1.38x (NIH, Bethesda, Maryland, USA) and converted into diameters (mm). We use egg diameter as a measure of maternal energetic investment as it is positively related to yolk volume ($F_{1,225} = 300.36$, $P < 0.001$, $R^2 = 0.57$ Fig. S1, Supporting Information). *Egg size* is the average egg diameter within a female. Two levels of egg size variability were estimated using the coefficient of variation ($CV \sim (SD/mean) \times 100$): *within-female variability* (within-female egg size CV averaged across females in a sample) and *among-female variability* (CV of average egg size per female from the same sample). Ovaries were dissected out of preserved fish and weighed to the nearest 1 mg to estimate *gonad weight*. *Fecundity* was calculated by summing the number of mature eggs in both ovaries with eggs that had been expressed from the female for maturity determination and egg size measurements and is a proxy for the number of offspring produced.

Many studies of fish have shown that reproductive investment (e.g. fecundity, egg size) is positively correlated to female body size (e.g. Fleming & Gross 1990; Beacham & Murray 1993; Humphries 1995; this study Table S1, Supporting Information), and thus, among-population differences in body size could conceivably result in and/or

explain differences in reproductive investment. Many authors therefore standardize trait data to a common body size to account for these allometric relationships and to facilitate comparisons among samples independent of maternal body size (Fleming & Gross 1990; Tamate & Maekawa 2000). However, whilst this approach has merit in trying to elicit other potential causes for variation in reproductive investment, actual fitness is still dependent on the raw, untransformed traits. Also, the interrelationship among reproductive traits and body size in fish means that adaptation or plasticity in, for example, fecundity can be brought about not just through a trade-off with egg size but also an increase body size (Parker & Begon 1986; Bernardo 1996). Body size differences among populations are potentially a very real and important source of adaptive variation in reproductive investment. We therefore decided to analyse both the raw and standardized reproductive trait data along environmental gradients to gauge the relative importance of among-population body size differences. We calculated standardized traits as follows:

$$M_t = M_o \times (L/L_o)^b$$

where M_t is the transformed character, M_o the observed character, L overall mean length (52.52 mm), L_o the observed length for each individual and b the regression coefficient of $\log(M_o)$ on $\log(L_o)$. There was some heterogeneity among populations in the within-population slopes of $\log(M_o)$ on $\log(L_o)$, so b is the common within groups slope obtained from ANCOVA (Reist 1986) and is 0.119 for egg size, 2.699 for fecundity and 3.280 for gonad weight.

HYDROLOGICAL VARIABLES

We developed a series of hydrological indices to describe the quality, variability and predictability of each stream environment. *Mean daily*

flow (mega litres ML) and the average number of days each year of zero flow both capture different aspects of environmental quality. Increasing mean daily flow equates to more water in a stream (at least in the types of streams we studied) and is related to the amount and type of habitat available (Bond & Lake 2003; Tonkin, King & Mahoney 2008) and potentially predation risk (e.g. Reznick, Butler & Rodd 2001). Zero flow is related to deteriorating physiochemical conditions (Magoulick & Kobza 2003; McMaster & Bond 2008), reduced habitat connectivity (Cook, Bunn & Hughes 2007; Perry & Bond 2009) and potentially reduced food availability (Boulton & Lake 1992; Clarke *et al.* 2010). The among-year variability in annual flows (annual flow variability) and components of zero flow give an indication of how variable an environment is from 1 year to the next and are measures of habitat permanence (Perry & Bond 2009; Kennard *et al.* 2010). Colwell's predictability index (environmental predictability) is the sum of two separate components: constancy (degree to which a state stays the same) and contingency (measure of how closely different states correspond to different time periods) (Colwell 1974). We calculated environmental predictability using monthly mean daily flow to measure the relative certainty of knowing a flow at a particular time.

High-quality flow gauge data (Victorian Water Resources Data Warehouse <http://www.vicwaterdata.net>) were available for six of the ten streams sampled. Modelled flows were used for the remaining four sites (Table 1) (Bond *et al.* 2011). Mean daily flow, zero flow and annual flow variability were estimated across the flow record (> 15 years, Table 1) as this was the only temporal scale available for modelled parameter estimates. Environmental predictability estimation requires a time series and was therefore only calculated for the six sites where gauge data were available. Hydrological analyses of gauge data were performed using the Time Series Analysis Module of the RIVER ANALYSIS Package (v3.0.3; eWater CRC, Canberra, ACT, Australia, Marsh, Stewardson & Kennard 2003) with Colwell's Predictability calculated using 11 classes with a logarithmic base of 2 (values recommended for flow data from south-east Australia).

DATA ANALYSIS

Analyses were performed in R 2.11.1 (R Development Core Team 2010). All data were checked for adherence to the assumptions of each statistical analysis, with the response variables egg size, fecundity, gonad weight and length subsequently natural log transformed for all tests. The trade-off between fecundity and egg size given finite resources was analysed using multiple regression.

EFFECTS OF ENVIRONMENTAL QUALITY AND VARIABILITY AMONG POPULATIONS

Among-population variation in reproductive traits (raw and standardized) was analysed using linear mixed effects models (NLME package 3.1-96) to account for the hierarchical nature of the data, potentially heterogeneous variances and temporal correlations (Zuur *et al.* 2009). For each trait model, the predictor variables, mean daily flow, zero flow and annual flow variability, were fitted as fixed effects (all continuous). Random effect structures of increasing complexity were fitted to the full additive fixed effect model using restricted maximum likelihood estimation (REML) and their relative support assessed using Akaike's Information Criterion corrected for small sample sizes (AICc; Burnham & Anderson 2002). First, a random intercept (population) was included to reflect the hierarchical nature of observations. Second, a temporal effect (day length in hours based on sample date and latitude, Table 1) identified repeated samples

within population and accounted for the tendency of trait values to decline through time within a season (Table S2, Supporting Information), and the possibility of mothers captured later in the season having already begun spawning. Third, we fitted an autoregressive correlation structure as replicate samples within a population are more likely to be similar than those from different populations. Lastly, we explored patterns of variance heterogeneity across population strata. The best random effect structures for each trait are presented in Table S3 and were adopted in the subsequent exploration of optimal fixed effect structures.

Seven models of increasing fixed effect complexity were fitted to each trait using maximum likelihood estimates of error and their performance compared using AICc. The optimal model was re-analysed using REML to produce unbiased parameter estimates (Zuur *et al.* 2009).

EFFECTS OF ENVIRONMENTAL PREDICTABILITY WITHIN AND AMONG MOTHERS

Sources of variation in egg size were explored using a random effects model (fitted with REML) with female (unique ID) nested within sample, which in turn was nested within population. Such a model structure enabled egg size variation to be partitioned to four levels: within a female, among females within a sample, among temporal replicates (sample) within a population and among populations. The importance of each variance level was ascertained by comparing the AICc of models with terms sequentially collapsed (Crawley 2007).

The relationships between both within-female variability and among-female variability and the predictor variables environmental predictability and egg size were explored using linear regressions. We consider our sampling approach as adequate to investigate among-generation bet-hedging despite it only taking a snapshot of egg size variation. Whilst within-female egg size variation decreases reproductive success in any one generation, it also decreases variability among generations. Among-generation bet-hedging occurs when mothers in a given reproductive event produce offspring with a range of phenotypes (in this case egg size) that experience the same environment; fitness benefits arise because some offspring phenotypes will be suitable for local conditions (Crean & Marshall 2009).

Results

There was considerable variation in reproductive traits among females and across samples (Table S1, Supporting Information). Individual eggs ranged in diameter from 0.91 to 1.55 mm, fecundities from 40 to 2651 and gonad weights from 17 to 1139 mg. Female lengths ranged from 33 to 85 mm. Given finite resources, fecundity \sim gonad weight/egg size. We identified this trade-off for all females ($F_{2,227} = 504.2$, $P < 0.001$, $R^2 = 0.816$), with fecundity positively related to gonad weight ($\beta = 0.837$, $P < 0.001$) and negatively related to egg size ($\beta = -1.688$, $P < 0.001$).

AMONG-POPULATION ENVIRONMENTAL QUALITY AND VARIABILITY EFFECTS

The results of model selection for among-population variation in reproductive traits along hydrological gradients are presented in Table 2 and the most parsimonious models in

Table 2. Results of model selection procedure for seven combinations of explanatory variables fitted to reproductive trait data

Model	df	AIC	AICc	Δ AICc
ln (length)				
MDF + AnnVar	7	-253.20	-252.83	0.00
MDF + AnnVar + ZF	8	-253.26	-252.78	0.04
ln (egg size)				
AnnVar	15	-936.88	-989.94	0.00
MDF + AnnVar	16	-991.45	-989.43	0.52
ln (fecundity)				
MDF + AnnVar	7	408.59	409.08	0.00
ln (gonad weight)				
MDF + AnnVar	7	477.74	478.22	0.00
MDF	6	477.91	478.27	0.05
MDF + ZF	7	479.11	479.60	1.38
AnnVar	6	479.44	479.80	1.58
ZF	6	479.55	479.91	1.69
Standardized egg size				
MDF + AnnVar	7	-969.28	-968.88	0.00
Standardized fecundity				
MDF + ZF	7	2966.79	2967.28	0.00
ZF	6	2967.01	2967.37	0.09
MDF + AnnVar + ZF	8	2968.57	2969.20	1.92
AnnVar	6	2967.01	2967.37	1.95
Standardized gonad weight				
ZF	14	-690.79	-688.92	0.00
AnnVar + ZF	15	-690.72	-688.57	0.35
MDF	14	-689.18	-687.30	1.62

AICc, Akaike's Information Criterion corrected for small samples size; Δ AICc, AICc differences.

Shown are models with similar levels of support (Δ AICc < 2, Burnham & Anderson 2002). MDF, mean daily flow; AnnVar, annual flow variability; ZF, zero flow days.

Table 3. *Egg size* was negatively related to increasing *annual flow variability* whilst *fecundity* was positively related to increasing *annual flow variability* (Fig. 2a,c; Table 3). *Length* and *fecundity* both increased with *mean daily flow* (Fig. 2e; Table 3). The lack of any relationship between *gonad weight* and hydrology (Table 3) suggests *egg size* and *fecundity* trade-off along a gradient of temporal environmental variability whereby mothers inhabiting more variable environments are allocating their finite resources to producing a greater number of smaller eggs.

Standardized egg size was also negatively related to *annual flow variability*, but *standardized fecundity* was not related to *mean daily flow* and, instead, was positively related to *zero flow* (Fig. 2b,d; Table 3). These discrepancies between raw and standardized data for *fecundity* highlight the importance of *length* differences in determining among-population *fecundity* variation. The positive correlation between zero flow and annual flow variability ($r = 0.83$) suggests that the egg size: egg number trade-off among populations along a gradient of environmental variability likely still occurs irrespective of *length* differences, a proposition supported by continuing lack of relationships between hydrology and *standardized gonad weight* (Table 3).

Table 3. Parameter estimates and test statistics from best models describing among-population variation in raw and standardized reproductive traits along hydrological gradients

Parameter	Estimate (SE)	df	F stat	P value
Raw data				
ln (length)				
MDF	0.002 (0.0008)	1,7	7.772	0.027
AnnVar	0.450 (0.224)	1,7	4.981	0.061
ln (egg size)				
AnnVar	-0.212 (0.064)	1,8	10.823	0.011
ln (fecundity)				
MDF	0.008 (0.003)	1,7	8.768	0.021
AnnVar	2.112 (0.788)	1,7	72832	0.031
ln (gonad weight)				
MDF	0.006 (0.003)	1,7	3.445	0.106
AnnVar	1.329 (0.956)	1,7	1.935	0.207
Standardized data				
Standardized egg size				
MDF	-0.0007 (0.0004)	1,7	4.351	0.075
AnnVar	-0.377 (0.097)	1,7	15.187	0.006
Standardized fecundity				
MDF	0.809 (0.579)	1,7	1.951	0.205
ZF	3.253 (1.131)	1,7	8.267	0.024
Standardized gonad weight				
ZF	-0.0007 (0.006)	1,8	2.378	0.162

MDF, mean daily flow; AnnVar, annual flow variability; ZF, zero flow days.

ENVIRONMENTAL PREDICTABILITY EFFECTS

The random effects model identified considerable variation in *egg size* within females (9.74%), among females within a sample (24.41%), temporally replicated samples within a population (22.10%) and among populations (39.75%). In general, females inhabiting more unpredictable environments produced more variable egg sizes ($F_{1,4} = 11.813$, $P = 0.026$, $R^2 = 0.747$, Fig. 3). *Among-female variability* and *egg size* were not related to *environmental predictability* ($F_{1,4} = 0.543$, $P = 0.502$, $F_{1,4} = 0.066$, $P = 0.809$, respectively). Regressions of population-averaged *within-female variability* and *among-female variability* on *egg size* identified no significant relationships ($F_{1,8} = 0.132$, $P = 0.726$, $F_{1,8} = 0.362$, $P = 0.564$, respectively).

Discussion

There was considerable variation in *Nannoperca australis* egg size and egg number within females and among females from different populations. We propose that much of the phenotypic plasticity in *N. australis* reproductive investment represents adaptive trade-offs and bet-hedging in response to predictable and unpredictable environmental heterogeneity.

AMONG-POPULATION TRADE-OFFS IN EGG SIZE AND EGG NUMBER

Contrary to the predictions of many theoretical models that assume a positive asymptotic offspring size: performance

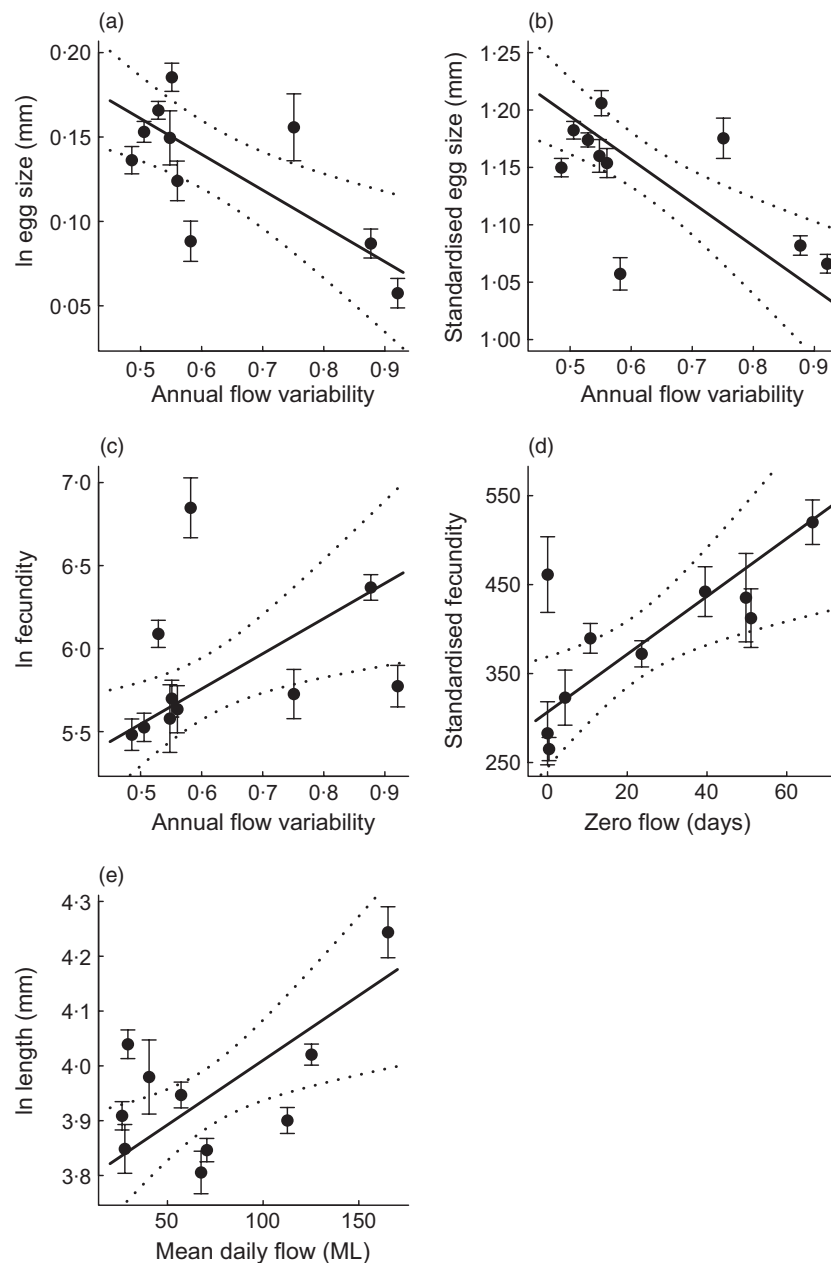


Fig. 2. Among-population variation in raw and standardized traits along hydrological gradients. (a) egg size, (b) standardized egg size and (c) fecundity vs. annual flow variability; (d) standardized fecundity vs. zero flow and (e) length vs. mean daily flow. Points represent observed population averages (± 1 SE), and lines predicted relationships ($\pm 95\%$ confidence intervals) between x and y variables with all other covariates at mean values.

function (Smith & Fretwell 1974; McGinley, Temme & Geber 1987; Forbes 1991; Lalonde 1991; Einum & Fleming 2004), we found that the average size of *N. australis* eggs decreased and egg number increased with increasing environmental variability and decreasing environmental quality. Our results, in this regard, are in concordance with Hendry, Day & Cooper's (2001) model predictions, based on a peaked fitness function, of bigger optimal egg sizes in better habitats and highlight the need to carefully consider the nature of offspring size: performance relationships. We propose that in *N. australis*, random mortality associated with periodic physical disturbance (in these systems caused by drought) may nullify the maternal fitness benefits associated with large offspring in some habitats. Instead, when offspring performance is random with respect to size (because of size-independent

causes of mortality), maternal fitness will be optimized through increasing fecundity.

Small streams in south-east Australia experience a high risk of drought-induced catastrophic habitat loss characterized by prolonged periods of zero flow and subsequent channel drying, and these events likely have a strong selective influence on fish populations (sensu Schlosser 1990). Often, the only suitable habitats for fish like *N. australis* are isolated pools that can be separated by tens of kilometres of dry stream channel (Bond & Lake 2003; McMaster & Bond 2008; Perry & Bond 2009). Whilst the higher fitness of larger *N. australis* larvae (survivorship under physiochemical stress and swimming speed, Morrongiello 2011) will manifest in wet years, any maternal or offspring fitness benefit is lost if the pool in which these individuals reside dries up.

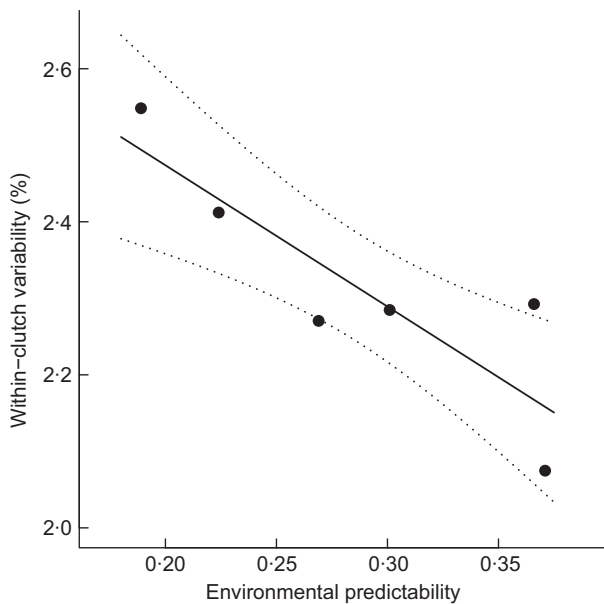


Fig. 3. Relationship between within-female egg size variation and environmental predictability as measured using Colwell's index. Lines represent fitted relationship and 95% confidence intervals.

Environmental filtering during the relatively frequent droughts will be strong enough to offset the fitness advantages associated with larger offspring. In systems that are highly ephemeral and drought prone (increased *annual flow variability* and *zero flow*), maternal fitness will be maximized by producing many small offspring that can spread throughout the stream before it ceases to flow. Such a reproductive tactic increases the likelihood that some offspring will disperse and find permanent water. Conversely, in the more perennial systems studied (lower *annual flow variability* and *zero flow*) where habitat is relatively permanent, abiotic stressors more benign and competitive interactions stronger, larger offspring with their associated fitness benefits would be favoured and fewer eggs produced.

Similar departures from an expected positive, asymptotic, relationship between offspring size and performance have been found in a range of animals. For example, Moran & Emlet (2001) found that in the intertidal gastropod, *Nucella ostrina*, larger hatchlings had higher fitness in relatively benign conditions, but these benefits decreased under high heat stress. Smaller juvenile lemon sharks (*Negaprion brevirostris*) had higher survivorship than larger juveniles, potentially because of predation-mediated selection against large size and fast growth (Dibattista *et al.* 2007), and oriental firebellied toad (*Bombina orientalis*) tadpoles originating from larger eggs were more likely to be eaten than those from smaller eggs when raised in warm ponds because of diminished locomotory ability associated with larger amounts of yolk (Kaplan 1992). In the bryozoan, *Bugula neritina*, offspring size was unimportant in determining fitness in both benign and extremely competitive environments, but larger larvae had higher performance at intermediate levels of competition (Allen, Buckley & Marshall 2008). It was suggested that in harsh environmental conditions, *B. neritina* maternal fitness

is maximized by producing offspring smaller than those favoured under more intermediate stress. Brockelman (1975) supports this proposition by stating that high birth rates (fecundity) and smaller optimal offspring sizes could eventuate as a consequence of high mortality rates if subsequent reductions in competition increase the fitness of smaller individuals. Our study provides further evidence to support the 'bigger is not always better in harsh environments' argument.

Although not tested in this study, it is possible that geographical differences in predation or competition regimes may be driving the observed among-population egg size and egg number variation. For example, the extensive work documenting life-history evolution in guppies (*Poecilia reticulata*) (e.g. Reznick, Rodd & Cardenas 1996; Reznick, Butler & Rodd 2001) has shown that mothers exposed to high predation regimes produce more numerous and smaller offspring, whilst larger offspring can have increased fitness in competitive environments (Brockelman 1975; Bashey 2008). However, in *N. australis*, we believe biotic factors to be of secondary importance to hydrological factors in driving spatial reproductive investment variation. Given that *N. australis* predation risk is likely to be higher in larger streams (Lake 1982; Schlosser 1987, 1990; J.R. Morrongiello pers. obs.), it might be expected that females inhabiting streams with higher *mean daily flow* and generally lower *annual flow variability* would produce more and smaller eggs if predation were important (Reznick, Rodd & Cardenas 1996). In fact, we observed the opposite pattern. An increased prevalence of *zero flows* can be associated with reduced food availability in ephemeral streams (Boulton & Lake 1992; Clarke *et al.* 2010), and this has the potential to result in increased competition for resources and thus favour the production of fewer, larger eggs in more ephemeral streams. Again, the opposite pattern was observed in the current study. Nonetheless, we acknowledge that biotic factors, additively or in synergy with abiotic factors, may explain some of the spatial reproductive investment variation observed in *N. australis* and warrant further exploration.

Selection favouring variation in other offspring phenotypes rather than just offspring size has long been accepted in the plant literature. For example, individual annual plants inhabiting unpredictable environments can produce seeds with different germination phenotypes, thus spreading the risk of complete reproductive failure because of poor conditions over multiple years (Cohen 1966; Childs, Metcalf & Rees 2010). Similar selection patterns may also occur in animals, whereby producing offspring with variable foraging behaviours (e.g. great tits *Parus major*; van Overveld & Matthysen 2010) or dispersal capabilities (e.g. Opisthobranch sea slugs; Krug 2009) may be favoured under conditions of resource limitation and environmental heterogeneity. Further exploration of these possibilities will complement existing work focussing on offspring resource allocation.

As egg size, fecundity and body size are intertwined by adaptive and constraining relationships (Bernardo 1996; Wootton 1998), it is worth considering both raw and

standardized data to help elucidate the causality of patterns observed. Interestingly, average female body size was positively correlated to *mean daily flow*. Variation in body size can be adaptively related to environmental conditions (Teder, Tammaru & Esperk 2008), so the pattern observed in our study may reflect adaptive plasticity in *N. australis* to increased flow and a means through which to alter fecundity or egg size (Bernardo 1996). Indeed, raw fecundity was positively related to *mean daily flow*, reflecting the strong influence of length on this trait (Table S2, Supporting Information). As expected, the influence of length on fecundity disappeared in the standardized data. In contrast, the similarities between raw and standardized egg size patterns suggest that egg size variation on all scales is largely independent of body size.

DIVERSIFIED BET-HEDGING AND WITHIN-FEMALE EGG SIZE

Of equal interest to patterns in average egg size along environmental gradients is how resources are allocated to offspring within a female. Female *N. australis* inhabiting increasingly unpredictable environments produced eggs of more variable size, providing empirical support for Marshall, Bonduriansky & Bussiere's (2008) model that stipulates diversified bet-hedging in egg size will prevail as an adaptive strategy when the state of future offspring environments is unclear. Similar patterns of within-female egg size variation indicative of diversified bet-hedging have been recorded elsewhere, including in brook trout (*Salvelinus fontinalis*) (Koops, Hutchings & Adams 2003), common jollytails (*Galaxias maculatus*) (Semmens 2008) and quacking frogs (*Crinia georgiana*) (Dziminski, Vercoe & Roberts 2009).

Koops, Hutchings & Adams (2003) hypothesized, based on Lalonde's (1991) predictions for iteroparous organisms, that within-female variability will be negatively related to average egg size when mothers have imperfect information about the future environment. Variable egg size helps offset some of the cost of producing small eggs when an environment is poorer than predicted. Within-female variability was not related to average egg size in *N. australis*, but such patterns have been documented in other fishes (Koops, Hutchings & Adams 2003; Semmens 2008). Similar to Koops, Hutchings & Adams (2003), we found no relationship between average egg size and environmental predictability, although average egg size was negatively related to environmental variability. The discrepancy between our results and those of others may be due to a number of reasons including: many *N. australis* populations being essentially semelparous, the tendency for *N. australis* to produce smaller eggs rather than larger eggs in poor-quality environments, variable offspring size: performance functions or because both good- and poor-quality environments can be unpredictable.

We found no evidence that *N. australis* among-female egg size variation, within a population, is related to environmen-

tal predictability. Kaplan & Cooper (1984) proposed that when mothers are uncertain about the future environment, among-female egg size variation will tend to increase, reflecting differences in the perception of optimal egg size and the adoption of a 'coin-flipping' strategy to determine offspring trait distributions. Kaplan & Cooper's (1984) model does, however, assume an even allocation of resources among eggs within a female that was not found in *N. australis*. In contrast, comparative studies of marine invertebrates (Marshall, Bonduriansky & Bussiere 2008) and fishes (Einum & Fleming 2002), as well as more recent simulation models (variable within-brood offspring size: Marshall, Bonduriansky & Bussiere 2008; constant within-brood offspring size: Fischer, Taborsky & Kokko 2011), have found that among-female variability in egg size increases in more predictable environments or when the maternal phenotype can influence the environment offspring experience through oviposition selection. These authors propose that as environments become more predictable, individual mothers should actively adjust offspring size to reflect different allocation optima depending on local conditions.

We propose that *N. australis* are adopting a diversified bet-hedging strategy in unpredictable environments. Alternative non-adaptive explanations such as physiological constraints on equal investment and maternal phenotype effects may, however, be invoked to explain some of the observed pattern in within-female variability. It may be possible that offspring are experiencing sibling competition (Parker & Begon 1986; Einum & Fleming 2002) as this can still occur in free-spawned eggs (Kamel, Grosberg & Marshall 2010), or that mothers significantly influence offspring environment (van den Berghe & Gross 1989). However, we believe these factors to be less important as *N. australis* do not build a nest, and larvae only develop for a short time in the egg when oxygen competition would be high, although siblings may compete for food once feeding commences. It is also unlikely that within-female egg size variation is because of mothers 'rounding' clutch size to the nearest integer as occurs in small (1–10) litter sizes (Charnov & Downhower 1995; Guinnee, West & Little 2004), as average *N. australis* fecundity is considerably larger (423 in this study). Similarly, whilst variation in maternal condition may be a physiological constraint on equal investment (Fox & Czesak 2000; Einum & Fleming 2004), there was little temporal difference in female body size through a season (Table S2, Supporting Information), and larger mothers were not better conditioned than smaller mothers (J.R. Morrongiello, unpublished data). Nonetheless, the development of offspring size: performance functions together with experimental manipulation of offspring environments and an appreciation of trait covariation are required to fully explore our contention (Childs, Metcalf & Rees 2010).

SYNTHESIS AND CONCLUSIONS

The form and parameterization of the offspring size: performance function plays a vital role in determining

expected maternal investment strategies and tactics in variable and unpredictable environments. The presence of both among-population and within-female egg size variation in *N. australis* likely reflects adaptive plasticity and bet-hedging in response to both predictable and unpredictable environmental heterogeneity. Our field-based results suggest that *N. australis* populations may be represented by a series of spatially and temporally varying peaked performance curves of increasing right skewness (decreasing mean optimal offspring size) or peak width (increasing random mortality) with increasing environmental harshness (among-population adaptive plasticity sensu Hendry, Day & Cooper 2001), and a parallel decrease in kurtosis along a gradient of decreasing environmental predictability (within-female diversified bet-hedging sensu Marshall, Bonduriansky & Bussiere 2008). Recent theoretical models have incorporated some, but not all, of these elements (e.g. Hendry, Day & Cooper 2001; Marshall, Bonduriansky & Bussiere 2008; Fischer, Taborsky & Kokko 2011), and we suggest that models be extended to account for the possibility of both predictable and unpredictable environmental variance being present and concurrently influencing fitness at the among-population and within-individual scales.

An evolutionary perspective can provide valuable ecological insights into the interaction between a species and its environment (Lytle & Poff 2004). Our data show that egg size and egg number can adaptively respond to both predictable and unpredictable environmental variation. Reproductive trait variation likely has important fitness implications for the individual that will in turn affect the resistance or resilience of populations to changing environmental conditions such as those brought about by natural perturbations (Lake 2003) or human-induced modifications to natural flow regimes (Poff *et al.* 1997).

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Supporting information

The following Supporting information is available for this article online.

Fig. S1. Relationship between egg diameter and yolk volume.

Table S1. Sample-specific trait data collected for this study.

Table S2. Spatial and temporal summaries of results presented in Table S1.

Table S3. Random effects structures used for each reproductive trait model.

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