

Atherton, J. A. and McCormick, M. I. 2017. Kin recognition in embryonic damselfishes. – Oikos doi: 10.1111/oik.03597

Appendix 1

Table A1. Mean mass (mg \pm SE) of eggs from three species of damselfish, calculated from five replicated measurements of dry weight, and resultant embryo alarm odour concentrations (\pm SE).

Species	Number of eggs	Mean mass (mg)	Mean alarm odour concentration (mg ml ⁻¹)
<i>Acanthochromis polyacanthus</i>	5	40 \pm 0.77	8.0 \pm 0.15
<i>Amphiprion melanopus</i>	10	36 \pm 0.70	7.2 \pm 0.14
<i>Chrysiptera cyanea</i>	63 (mean)	38 \pm 0.61	7.6 \pm 0.12
Overall mean	N/A	38 \pm 0.70	7.6 \pm 0.14

Appendix 2

Pilot trial

A pilot trial was conducted to determine if it was possible to freeze embryos for later use as alarm odour donors in embryo behaviour trials. This would allow for a wider range of samples to be tested, such as testing recently produced embryos with clutches previously produced by the same breeding pair.

The experiment used *Amphiprion melanopus* and *Acanthochromis polyacanthus* embryos to compare the reactions to either an alarm odour produced from fresh embryos or embryos frozen in liquid nitrogen, or a seawater control. Embryos were sourced from clutches produced by three separate breeding pairs, for each species, with fifteen embryos from each being tested against one of the three chemical cues. A linear mixed-effects ANOVA model tested odour type (fresh or frozen) and test species as fixed factors, but also included clutch as a random factor.

The results demonstrated that alarm odours created from frozen (and defrosted) embryos produced a very similar level of response to fresh alarm odours (Tukey's HSD: $p = 0.59$; Fig. A1, Table A2). Hence, it was deemed acceptable to use alarm odours produced from frozen embryos.

Table A2. A two-factor fixed ANOVA comparing the change in heart rate induced by alarm odours produced from fresh, or frozen and defrosted, embryos, which was crossed with the species that was tested.

Effect	df	MS	F	p
Odour	2	3354.18	162.981	< 0.0001
Species	1	2.70	0.131	0.7177
Odour × Species	2	4.39	0.214	0.8079
Residual	264	20.58		

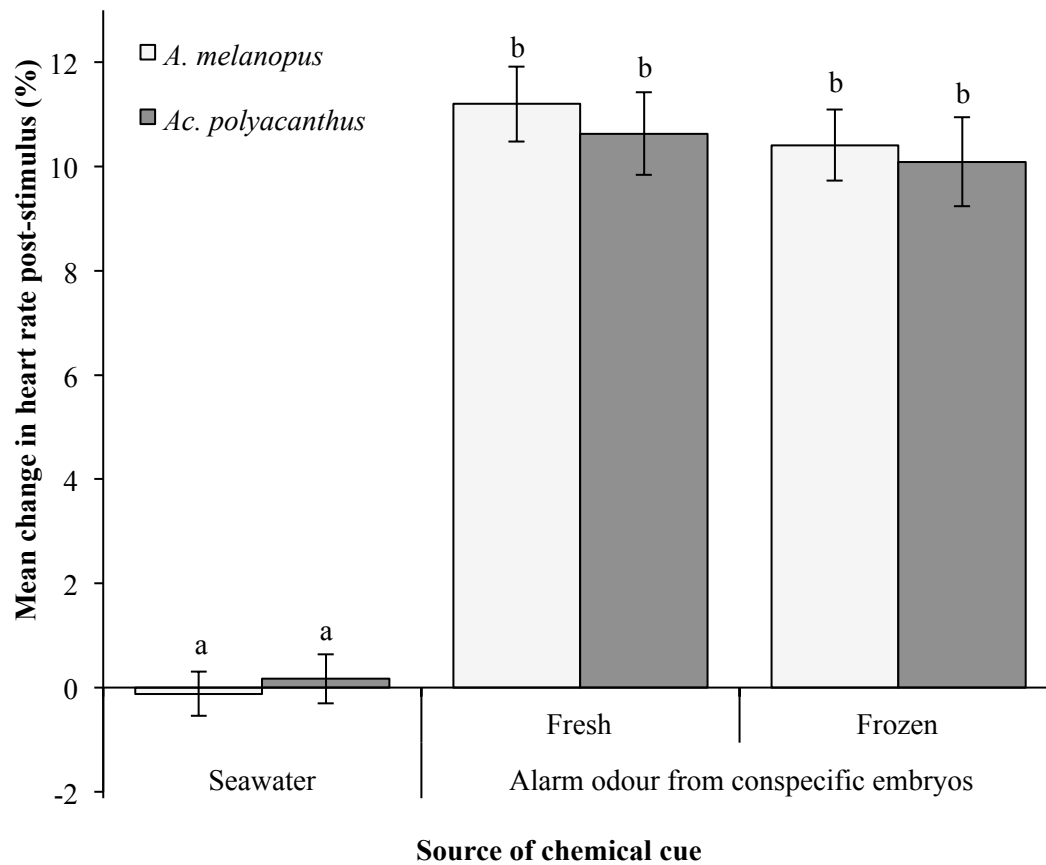


Figure A1. Comparison of the mean percentage change in heart rate (\pm SE) produced by the introduction of a seawater control, or an alarm odour produced from either fresh, or frozen and defrosted, conspecific embryos (for each bar $n = 45$). Letters denote Tukey's groupings of means.

Appendix 3

Table A3. Sample sizes, in terms of the number of embryos and clutches tested for each chemical cue for both *Amphiprion melanopus* and *Acanthochromis polyacanthus*. The number of embryos from each species tested for each odour was opportunistic and dependent upon the reproductive success of the breeding pairs and the availability of embryos from which the alarm odours were created.

Chemical cue type	<i>A. melanopus</i>		<i>Ac. polyacanthus</i>	
	Embryos	Clutches	Embryos	Clutches
Seawater	75	5	120	8
Kin	75	5	120	8
Kin previous	45	3	30	2
Non-kin	45	3	75	5
<i>Ac. polyacanthus</i> / <i>A. melanopus</i>	45	3	55	4
<i>C. cyanea</i>	45	3	30	2