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An experimental investigation of salinity effects on growth, development and condition in the European flounder (*Platichthys flesus*. L.)

Bernadette O'Neill *, Fien De Raedemaecker, David McGrath, Deirdre Brophy

Marine and Freshwater Research Centre, Department of Life Science, Galway-Mayo Institute of Technology, Dublin rd, Galway, Republic of Ireland

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ABSTRACT

European flounder (*Platichthys flesus*. L.) is a euryhaline flatfish species which can actively migrate towards and cope with low salinity environments. A laboratory experiment was undertaken to analyse the effect of salinity on condition and growth of metamorphosing European flounder. The working hypothesis was that flounder, which preferentially settle in low salinity habitats, would display accelerated development and/or enhanced growth and condition at lower salinities. The fish used in the experiment were in the late stages of metamorphosis. At the end of the 21 day laboratory rearing period no significant difference in ontogenetic development was found between exposures (salinity of 0, 10, 20 and 30). No significant differences in somatic growth rate, somatic condition or standard length were observed between treatments. There was no correlation between RNA:DNA ratio and somatic condition. Contrary to expectations, mean RNA:DNA ratios (measure of short-term well being) tended to increase with salinity and were significantly higher in the 30 salinity exposure compared to the 0 salinity exposure. The working hypothesis was, therefore, rejected. The results demonstrate that laboratory observations can fail to capture the complex ecological interactions at play in field environments. The preference for low salinity environments may be driven by other environmental factors such as predator/competition avoidance and food supply.

1. Introduction

Estuarine and shallow marine habitats are important nurseries for the larval and juvenile stages of many flatfish species (Cabral et al., 2007; Vasconcelos et al., 2011). Here species distributions overlap (Able, 2005; Ramos et al., 2009) and species specific microhabitat use can occur (Ramos et al., 2010). Estuarine nurseries can offer a number of advantages for young fish such as predator and competition avoidance, high food availability and rapid growth and development (Beck et al., 2001, 2003; Le Pape et al., 2003). However, estuaries can also impose physiological challenges on developing fish as the levels of certain abiotic factors can vary greatly between and within estuaries (Cabral et al., 2007). Whilst intermediary salinity conditions can infer an advantage in terms of growth (Boeuf and Payan, 2001), low and variable salinity conditions (due to river run off and tidal fluctuations) are a common feature of estuarine systems which can result in an increase in osmotic pressure (Hutchinson and Hawkins, 1990).

Salinity influences energy expenditure in fish; there is a significant energetic cost associated with the mechanisms used by fish to maintain osmotic balance (Boeuf and Payan, 2001). Osmoregulatory cost is generally lowest under isosmotic conditions (Jobling, 1994; Likongwe et al., 1996) and can increase when moving from stable to variable salinity environments (Hutchinson and Hawkins, 1990). However, an isosmotic environment is not preferential for all species where optimal salinity in terms of growth and condition can vary during ontogenetic development (Allen and Chec, 2007; Cardona, 2000; Partridge and Jenkins, 2002). From a review of the literature, Deacon and Hecht (1999) showed that in general, marine spawned fish grew better at salinities higher than the isosmotic level whilst fresh water spawned fish had optimal growth below the isosmotic level. Information on species specific salinity tolerances and their interaction with ontogenetic development is useful for maximising growth rates, condition and development rates in aquaculture whilst recognition of environmental conditions promoting enhanced growth, survival and recruitment can aid the identification of high-quality nursery habitats.

P. flesus is an estuarine dependant flatfish during its juvenile phase (Martinho et al., 2010) and can therefore be exposed to more variable abiotic factors compared to species in other coastal habitats. Benthic settlement concludes the pelagic larval phase (Van der Veer et al., 1991) and is associated with metamorphosis in the majority of flatfish (Geffin et al., 2007) Low salinities can induce immediate metamorphosis in mature *P. flesus* larvae (Hutchinson and Hawkins, 2004). In the absence of sufficiently low salinity, flounder may delay settlement prolonging their sensitive larval phase (Hutchinson and Hawkins, 2004). Once settled, the post larval flounder exhibit vertical migrations and use estuarine tidal stream transport (Bos, 1999; Jager, 1999), to actively migrate to oligohaline sites (Bos and Thiel, 2006). Kerstan (1991) found that within estuaries, densities of juvenile

* Corresponding author. Tel.: +353 917 425 02.
E-mail address: oneill.bernadette@gmail.com (B. O'Neill).

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P. flesus significantly increased with decreasing salinity. Salinity is therefore thought to be a steering factor in flounder transport, migration (Jager, 1998) and development (Hutchinson and Hawkins, 2004).

The overall quality of nursery areas can affect flounder growth, condition (Amara et al., 2009), survival and eventual recruitment to the adult populations (Power et al., 2000). Biochemical and morphometric indices are representative of the health and energy status of individual fish and are therefore reflective of overall habitat quality (Amara et al., 2009; Tanner et al., 2009; Vasconcelos et al., 2009). RNA:DNA ratios (a nucleic acid derived condition index) were first used to examine the nutritional status and general well being of fish in the 1960's and since then have become more widely used (Gilliers et al., 2004; Imsland et al., 2002; Mercaldo-Allen et al., 2006; Peck et al., 2003). This biochemical index reflects variation in protein synthesis rates where it is assumed that the amount of DNA (an index of cell number) is stable under changing environmental conditions whereas the amount of RNA (an index of the protein synthetic capacity of a cell) varies (Bulow, 1970, 1987).

Given that *P. flesus* migrate towards and develop in low salinity environments (Bos and Thiel, 2006; Hutchinson and Hawkins, 2004), it is hypothesised that post larval flounder will display enhanced growth, condition and rates of development under these conditions. Gutt (1985) showed that at a size of 4.2 to 5.3 cm (3–4 months after settlement) food conversion rates, growth and condition of *P. flesus* are highest at intermediate salinities. The aim of the present study was to assess the overall affect of salinity on the development, growth and condition of *P. flesus* during and shortly after metamorphosis under controlled experimental conditions. Somatic growth rates and RNA:DNA ratios are used as indices of individual condition. Although experimental studies are not truly reflective of the natural environment, the assessment of environmental variables in isolation may provide an understanding of their effects upon fish life history traits.

2. Materials and methods

2.1. Biological sampling

Post larval flounder were sampled during low tide from a known flounder nursery within Galway Bay on the 16th of April 2010. It is therefore assumed that all flounder sampled were from the same cohort. The site was situated within an estuary on the Oranmore River where salinity frequently falls below 0.5 salinity during low tide. Previous sampling expeditions recorded high abundances of flounder in this particular habitat where the presence of other flatfish species was rare. Flounder were sampled using hand nets. 200 fish were transferred alive to buckets containing water collected from the estuary whilst 40 fish (start control) were immediately frozen in liquid nitrogen on site. Subsequent to the experimental period fin rays of all fish were counted to confirm species identification (Russell, 1976; Wheeler, 1969). Due to the high mortality and stress associated with fish handling it was decided to use the 40 control fish as a base line for comparison with post experimental fish, using the increase in mean length and weight measurements to estimate growth.

2.2. Experimental design

Four separate blue plastic re-circulation tanks were used for each salinity exposure. Each tank contained four separate compartments and fish were completely contained within each compartment and were unable to move between compartments. Three compartments were used as treatment replicates where each replicate held ten fish and the fourth held extra flounder. Where mortality occurred within a treatment, replacement fish were added from the fourth compartment to maintain constant densities in each replicate. Initially the water in each tank (dechlorinated tap water) was held at 0 salinity which was comparable to their natural environment in the estuary. After a period of acclimatisation (7 days) each tank had its salinity increased steadily (over 5 days) by gradually adding a concentrated mixture of dissolved peacocks sea salt and dechlorinated tap water until the desired salinity exposure was reached. The treatment conditions during the 21 day experimental period were held at the follow salinities: Tank 1: 0; Tank 2: 10; Tank 3: 20; and Tank 4: 30. Temperature was maintained at around 11 °C which closely matched the temperature in the natural environment. All fish were killed in liquid nitrogen after the experimental period and were subsequently stored at −80 for further analysis. Standard length (SL) (mm) and weight (g) of all fish were measured.

2.3. Feeding and rearing conditions

Nutritionally deficient *Artemia* were enriched with a homemade enrichment procedure described by Tamaru et al. (2003). Post larvae were fed live enriched *Artemia* nauplii twice daily. *Artemia* concentrations were increased steadily as the fish grew, from 300 *Artemia*/fish/day to 500 *Artemia*/fish/day. The unit used to house the experiment was devoid of external/natural light, instead a simulated natural light regime 14L:10D was used. The water was gently aerated and nitrite, ammonia, salinity, temperature, pH and dissolved oxygen were monitored daily. A 15%–25% water change was carried out every 3–5 days to maintain ammonia, nitrite and pH levels.

2.4. Otolith and eye migration examination

Sagittal otoliths (n = 116) were removed from each fish and mounted in crystal bond. The presence/absence of accessory primordia (AP), which indicates the start of settlement (Karakiri et al., 1989) and metamorphosis (Modin et al., 1996), was assessed on the right otolith under a compound microscope at 200× and 400× magnifications. All fish examined (n = 116) displayed at least one AP on their sagittal otoliths. The ontogenetic stages of *P. flesus* were determined upon examination of eye migration, using appropriate keys (Hutchinson and Hawkins, 2004; Keefe and Able, 1993). All fish examined fell into three developmental categories: Stage IV, most of the left eye visible from the left side; Stage V, entire left eye is past the dorsal midline; and Stage VI, eye completely translocated.

2.5. Analytical protocol

RNA:DNA ratios were determined for each individual following a method described by Caldarone et al. (2001) and Clemmesen (1993). Essential trials were carried out (detection limits, standard calibration curves of RNA (Bakers yeast) and DNA (calf thymus) and spike recovery of homogenates) prior to routine use of the procedure as suggested by Caldarone et al. (2001). New standard curves were created for each 96 well plate.

Fish heads, caudal fins and gut contents were excised prior to analysis, therefore ensuring that gut content did not contribute to RNA:DNA ratio. Dissecting tools were rinsed with de-ionised water after each fish dissection to avoid cross contamination. Tissue was homogenised using glass beads and TEN-SDS 0.01% buffer and vortexed (15 min) using the pulse option and subsequently centrifuged (6000 rpm for 10 min at 4 °C). The supernatant was collected and stored in microtubes. Replicate samples of each supernatant were analysed to ensure acceptable reproductability. Nucleic acid concentrations were determined using an Ascent microplate fluorometer at the excitation wavelength of 355 nm and emission wavelength of 592 nm. Fluorescence was measured using ethium bromide. Total fluorescence was measured initially to determine total fluorescence within each sample. RNase (5 μl) was subsequently added to each