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Behavioural response to detection of chemical stimuli of predation, feeding and schooling in a temperate juvenile fish

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ABSTRACT

In order to recruit into adult populations juvenile fish must be able to find food, successfully compete with other organisms and avoid predation within a habitat, in other words they must be able to locate favourable and avoid detrimental conditions. Bio-chemical research into fish detection and discrimination between chemical cues is extensive, however whether olfactory mechanisms are critical in habitat selection and avoidance of detrimental conditions within the marine environment remains under-researched. Despite being one of the scientifically most explored seas, studies on the use of olfactory system in the selection of water masses of the Mediterranean fish species are absent. Using a chemical choice flume, the chemically mediated behaviour choices to distinct chemical cues (algae, seagrass, predator and conspecifics) of *Symphodus ocellatus*, a common Mediterranean fish, were investigated. In addition to the conventional analysis, which relies on the amount of the time spent in the specific water mass as the main indicator of preference, the behavioural response triggered by the detection of a particular cue was also examined, by analysing the mean and variance of speed of the individual fish movements, a complimentary approach previously not considered in the flume experiments. Bayesian statistical method was used to calculate both, proportion of time spent in the specific water mass, as well as to analyse the behavioural response of each individual within the specific water mass. In terms of the time spent, the flume trials conducted resulted in no significant fish selection preference or avoidance for any of the water masses tested, however varied speeds and number of burst speed movements were observed in a number of trials. When no olfactory stimulus was present, no change in behaviour was triggered. Thus, juvenile *S. ocellatus* undoubtedly has a capacity of change in behaviour to a complex array of olfactory stimuli, nevertheless the response in the flume experiment was more complex than just the differences in time of the occupancy between the water masses. As a result, the analyses of speed could in future prove to be an important complementary tool for studying behavioural responses of fish using this methodology. The findings are coupled with the development of the rigorous novel protocol for behavioural analyses using exclusively publicly available apparatus and software, all described within the manuscript.

1. Introduction

Settlement and post-settlement processes play a fundamental role in the maintenance of adult fish populations in the marine environment (Hixon, 1998; Jones, 1991). After spawning and fertilisation of eggs the surviving larval stages of most demersal species of fish generally remain in the water column to complete part or the entire larval development phase (Hannan and Williams, 1998; Jenkins et al., 1998). During this pelagic stage larvae are dispersed by currents, followed by settlement onto a benthic habitat and subsequent development into juveniles (Thresher et al., 1989). Several factors determine how suitable the benthic habitat is for the newly settled juveniles, because once settled they face a number of challenges. In order to ensure future recruitment into adult populations fish must be able to find food, successfully compete with other organisms and avoid predation within the settlement habitat (Beck et al., 2001). Accordingly, locating microhabitats in which those processes are optimised, as well as successfully recruiting to other habitats during subsequent life stages, is highly advantageous in terms of the growth and survival (Igulu et al., 2011). Thus, fish fitness and survival partly depend on the ability to acquire information from the

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environment in order to orientate towards the suitable habitat. In the ocean, this information can be acquired through a number of environmental cues, including biotic and abiotic, which subsequently guide a fish through different habitats with the aim of reaching the desired one (Huijbers et al., 2012).

Guidance mechanisms which rely on the detection of environmental cues, and thus facilitate successful movement between locations and habitats, may utilise acoustic, visual and chemical cues via specifically adapted sensory systems (Paris et al., 2013; Staaterman et al., 2012). A number of studies have identified the attraction of fish to biological reef noise (Leis et al., 2003; Simpson et al., 2004). In addition, it has been discovered that different components of the acoustic signature of a reef are important at different life stages, suggesting that the stimulus may also change with ontogeny. Juveniles and adults are attracted to low-frequency fish sounds (Simpson et al., 2008), while the settlement stage fish respond to the higher frequency invertebrate crackle (Simpson et al., 2004). These auditory cues can be detected at long distances, highlighting their importance in guiding directional movement towards a habitat (Radford et al., 2011; Simpson et al., 2010). On the other hand, visual cues are used by fish over short distances for microhabitat selection during settlement, and to some extent over the subsequent demersal life cycle for foraging, reproduction and anti-predator behaviour (Guthrie et al., 1993; Igulu et al., 2011). The use of the visual sensory system is of particular importance in areas of high water transparency (McCormick and Manassa, 2008). Predator avoidance, directed searching and prey location beyond visual range are however possible through chemical detection using the olfactory system (Atema, 1980). Moreover, many studies suggest that olfaction may be the most important cue for locating habitats over both large and small spatial scales (Baird et al., 1996; Dittman and Quinn, 1996; Lecchini et al., 2005a). The olfactory system in fish is highly sensitive and is, in terms of organisation, similar to that of the higher vertebrates, with some species having the ability to sense chemicals in the water at concentrations as weak as 10^{-9} M (Hara, 1992).

Throughout the last decade, a number of studies have been published addressing the topic, and despite this effort, the question whether olfactory mechanisms are critical in habitat selection remains under – researched (Coppock et al., 2013). On the other hand, bio-chemical research into fish detection and discrimination between chemical cues is extensive. The many compounds involved have been known for a long time and range from simple amino acids to complex mixtures of molecules from both biological and environmental origin. Compounds such as pheromones, peptides, amino acids, proteins, lipids and several other products of decomposition have been identified among the important ones (see Hara, 1992 and Derby and Sorensen, 2008). Other compounds which can be detected and may produce a response in fish include mannitol from algae, tannins from terrestrial plants and anthropogenic pollutants including industrial waste, sewage and insecticides (Dixson et al., 2010; Havel and Fuiman, 2015; Kingsford and Gray, 1996). Fish are also able to respond to specific chemical signals from coral tissue and conspecifics, symbiotic partners and predators (Arvedlund et al., 1999; Lecchini et al., 2005b, 2005c; Sweatman, 1983). Additional effort is required to fill the gap between the knowledge of the presently known chemical stimulants and their influence on fish habitat selection.

Within the Mediterranean Sea, seagrass meadows, erect macroalgae forests covering rocky reefs and shallow heterogeneous mixed bottoms composed of sand, gravel, pebbles and rocks constitute the most important sublittoral habitats in terms of juvenile fish development (Garcia-Rubies and Macpherson, 1995; Harmelin-Vivien et al., 1995; Moranta et al., 2006). Seagrass meadows are formed by a number of seagrass species, however *Posidonia oceanica* is the most dominant in the region (Larkum et al., 2006). Within sublittoral habitats of the Mediterranean macroalgae forests that form on rocky substrata are dominated by Fucales, mainly of the genus *Cystoseira* (Ballesteros et al., 1998). Seagrass meadows and the macroalgae forests are highly structurally complex and are considered key ecosystem engineers (Coleman and Williams, 2002). Although one of the most heavily researched seas, studies on the use of olfactory system in the selection of water masses of the Mediterranean fish species are absent. In fact, at present, very little is known about the ecology of Mediterranean juvenile fishes, despite their importance in the maintenance of system biodiversity and productivity (Halpern et al., 2005). Essential habitats for juveniles are not accurately defined and factors influencing their selection within the Mediterranean are poorly understood (but see studies by Guidetti, 2000; Cheminée et al., 2013).

By deploying the flume-based design, which over the past two decades has been highly popular in habitat selection studies (Atema et al., 2002; Coppock et al., 2013; Dixson et al., 2008; Gerlach et al., 2007 and references therein), an attempt was made to determine the effects of chemical cues on water mass selection or avoidance in juvenile *Symphodus ocellatus*, an abundant fish species resident within the Mediterranean. To date however, in order to test for water mass selection, the flume methodology studies exclusively rely on the amount of the time spent in the specific water mass and use it as the single indicator of the preference (e.g. Coppock et al., 2013; Dixson et al. 2011, 2008; Munday et al., 2009). As a result, an effort was also made to demonstrate that the responses of fish in the flume experiments can be more complex than just the difference in time of the occupancy between the two water masses and that perhaps, the somewhat simplistic belief that the behaviour can be described using a single indicator is not entirely true. Thus the behavioural response triggered by the detection of a particular cue was examined by analysing the mean and variance of speed of the individual fish movements, a complimentary approach previously not considered in the flume experiments. The following hypotheses were tested in the study: i) juvenile fish will display a preference for water containing chemical cues from conspecifics, as well as seagrass and macroalgae, when compared to seawater controls; ii) fish will avoid water containing predatory chemical cues; iii) the speed of response will be greater in juvenile fish exposed to water containing predator cues compared to habitat or conspecific cues, since the movement of fish was expected to be greater under stressful conditions. Finally, a more sophisticated Bayesian statistical method was used to calculate both, proportion of time spent in the specific water mass, as well as to analyse the behavioural response of each individual within the specific water mass. The statistical approach was coupled with the development of the rigorous novel protocol for behavioural analyses using exclusively publicly available apparatus and software, all described within the manuscript.

1.1. Material and methods

1.1.1. Study species

Wild type juveniles of the Ocellated wrasse (*Symphodus ocellatus* (Linnaeus, 1758)), between 1.4 cm and 3.6 cm standard length, were used in the experiments. This species is ubiquitously found in the Mediterranean and demonstrates a strong association with algal-covered rocky substrates as well as *P. oceanica* seagrass beds, throughout both juvenile and adult life stages (Cheminée et al., 2013 and references therein). The male builds, maintains and guards a nest made of algae in which several females lay their eggs (Whitehead et al., 1986). Newly hatched larvae have a pelagic larval stage of 9–11 days after which they settle on rocky bottoms covered with algae and *P. oceanica* beds, at a length of approximately 8 mm, where they remain permanently (Crec'hiriou and Lenfant, 2015; Raventós and Macpherson, 2001). Tests individuals were caught while SCUBA diving using hand-held nets, transported to the laboratory and acclimatised for a minimum of 24 h. The fish were held in 90 l, open circulation holding tanks at a stocking density of approximately 30 individuals per tank. The water supplying the tanks was sand filtered and UV sterilised seawater.