

**RESPIRATORY RESPONSE TO THE CHEMICAL CUES OF INJURED CONSPECIFICS
AND HISTOLOGY OF SKIN IN ROUND GOBY, *NEOGOBIOUS MELANOSTOMUS*
(ACTINOPTERYGII: PERCIFORMES: GOBIIDAE)**

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Pūtys Ž., Ložys L., Būda V. 2015. Respiratory response to the chemical cues of injured conspecifics and histology of skin in round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae). Acta Ichthyol. Piscat. 45 (4): 411–415.

Abstract. Round goby, *Neogobius melanostomus* (Pallas, 1814), responded significantly to conspecific skin extract by decreasing the frequency of opercular movements during the first minute of post-stimulus period, suggesting cryptic behaviour as antipredator reaction of this species. Examination of skin samples of the goby revealed the presence of club cells in the epidermis, the putative source of chemical cues that induce antipredator reactions in other fish species and may be the source of round goby injury-released alarm signals. Observed change in opercular movements is the first evidence of a round goby response to the chemical cues of injured conspecifics.

Keywords: anti-predator behaviour, club cells, gill ventilation rate, opercular beat rate

Early detection of the presence of predators is often the key to the survival of prey. Although visual signals during these interactions are often stronger and are transmitted more rapidly than chemical cues, the sense of smell is a very important source of information, including impending predatory attack, in aquatic ecosystems, especially under low-visibility conditions (Smith 1992). Chemical cues that signal alarm to prey species are very important for assessing the threat from predators, as well as facilitating cognitive and learning processes that give rise to behavioural, morphological, and life history adaptations (Ferrari et al. 2010). Specific forms of chemical information are characteristic for various predator–prey interaction stages such as detection, attack, capture, and ingestion (Chivers and Smith 1998). Alarm cues can be in the form of disturbance cues, diet cues, predator odours, and injury-released alarm cues (Chivers et al. 2007, Vavrek and Brown 2009, Maniak et al. 2000). Chemical alarm cues released following injury to prey, have recently become a widely and frequently studied topic. Various anti-predator behavioural reactions to chemical cues released by injured conspecifics are typical for most aquatic organisms, including many groups of fishes: cyprinids, salmonids, percids, catfish—both freshwater and marine (Chivers and Smith 1998, Ferrari et al. 2010). Response to injury signals has also been found among some gobies (Gobiidae) (Smith et al. 1991, Smith 1992, McCormick and Larson 2007, Marsh-Hunkin et al. 2013).

Round goby, *Neogobius melanostomus* (Pallas, 1814), originating from the Ponto-Caspian region, in-

vaded the Baltic Sea and the Great Lakes in North America during the last decade of the 20th century (see Kornis et al. 2012 for review). Goby populations can reach high densities, competing with native species for food and habitats, changing the dynamic balance of the ecosystem (Corkum et al. 2004). It is well documented that the round goby became an important food source for piscivorous predators in newly colonized territories (Bzoma and Meissner 2005, Pūtys and Zarankaitė 2010, Reyjol et al. 2010). Although reproductive strategy (e.g., parental care), diet breadth, or environmental tolerances were identified as the most important attributes for successful invasion (Garcia-Berthou 2007), ability to effectively avoid predators might be another important mechanism leading to successful establishment in new areas. This study investigated round goby responses to conspecific chemical cues released following injury.

Adult round gobies were collected in the northern part of the Curonian Lagoon (Lithuania) during October–November. Fish were held in a closed recirculating system and exposed to a natural photoperiod regime at the temperature of 17–18°C, ~ 7.6 pH and fed ad libitum once a day with frozen *Chironomus* sp. larvae. The total length (TL, cm, mean ± standard error of the mean) of round gobies exposed to skin extract (15.6 ± 0.9) and distilled water (15.4 ± 0.5) did not differ (Mann–Whitney *U* test, *P* = 0.57). Fish transportation, holding, and experiments were performed in accordance with the general guidelines for animal studies and applicable laws of Lithuania.

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The procedure for skin extract preparation was adopted from Brown et al. (2004). Skin extract was prepared using two donor fish (TL, 12.5 and 11.2 cm), killed with a sharp blow to the head. Skin fillets without fragments of muscle tissue were removed from both sides of the fish after making shallow cuts with a scalpel. Fillets of skin were measured using electronic callipers, placed in 50 mL of chilled distilled water, homogenized with a blender, and filtered. The filtrate was diluted with chilled distilled water to produce a solution of 10 mm² of skin per 1 mL of distilled water. The diluted filtrate was then immediately frozen in 1.5 mL aliquots and stored at -20°C prior its use in experiment.

Three fish were used for preparation of histological sections. Skin tissue with underlying musculature from the anterior dorsal area of the body was removed for preparation of histological sections and preserved in 10% formaldehyde. Skin samples were then mounted in paraffin wax blocks, sliced with a microtome to a thickness of 3 µm, stained with Schiff reagent, and counterstained with haematoxylin (PAS-H) (Ide et al. 2003). Stained sections were mounted on glass microscope slides and examined for presence of club cells under a compound light microscope at 200× magnification.

Rate of gill ventilation was studied as a potential response to conspecific skin extract because preliminary tests did not reveal locomotor reactions. Randomly selected fish were placed individually into non-recirculating 16 L glass aquaria containing 12 L of non-chlorinated tap water aerated continuously with an air stone. The rear and side walls of the test aquaria were covered with an opaque coating. A ceramic shelter (9 cm high, 18 cm long, and 6 cm wide) was placed into the aquarium. Twenty specimens (adult animals of both sexes) were used in the experiment, each fish was used for the test only once. Fish were fed before transfer to a test aquarium and left for acclimation per night for at least 16 h. The test aquarium and camera, used for recording the fish, were covered with an opaque screen to avoid disturbance of the fish. Gobies were exposed to concentrations of extract equating to 2 mm² skin per 1 L of water in the experimental aquaria, a concentration similar to that used in other experiments (Brown et al. 2004). An appropriate amount of prepared skin extract solution was diluted to 10 mL with distilled water and injected through a glass tube using a syringe, then 10 mL of distilled water was injected immediately afterwards so that all the solution would be flushed into the aquarium. The same experimental design was applied for the control using 20 mL of distilled water. 10 replicate trials with treatment and 10 replicate trials for control were conducted. Water parameters were measured immediately after each trial: temperature (mean ± SE) was 17.7 ± 0.1°C, dissolved oxygen concentration was 6.96 ± 0.07 mg·L⁻¹ and pH was 7.67 ± 0.05.

Fish were recorded for 5 min before and after adding skin extract or distilled water. The rate of gill ventilation was estimated by counting the number of opercular beats per 20 s interval (15 intervals before- and 15 after stimulus). A 5 min pre-stimulus period was recorded for

estimation of the basal opercular beat rate which was compared to the beat rate after the skin extract or distilled water was injected. Post-stimulus counting commenced 20 s after injection because observations using a dye prior to the experiment indicated that this period was required for the solution to become evenly distributed in the test aquarium.

The data were expressed as mean ± standard error of the mean (SE). The coefficient of variation (precision of the mean measured as the SE · mean⁻¹) among the observations for each individual fish during all time periods for both treatment and control results ranged from 0.01 to 0.05. As the opercular basal beat rate among individual fish differed, changes in the rate were expressed as relative change comparing to basal beat rate in every trial. Data were tested for normality using the Kolmogorov–Smirnov test. As most data were not normally distributed, the Mann–Whitney *U* test was used to compare change in gill ventilation rate between the distilled water controls and skin extract treatments at an a priori significance probability $P < 0.05$.

Examination of round goby skin samples revealed the presence of club cells in the epidermis (Fig. 1). Club cell cytoplasm did not react to PAS-H, becoming colourless, whereas mucous and surrounding epithelial cells are PAS-H positive and appeared dark; this test detects the presence of carbohydrates and the non-basophilic cytoplasm. These cells were located in the middle epidermal layer and were larger and differently shaped from surrounding cells. Club cells were singly dispersed in one or two layers, lacked pores connecting them to the skin surface, and their size ranged from 15 to 20 µm.

The rate of round goby gill ventilation did not change after exposure to distilled water during the control trials when compared with both the control period of 5 min ($P = 0.7$), and the 1 min time intervals ($P > 0.3$).

Gill ventilation rates among round gobies exposed to the skin extract decreased significantly ($P < 0.01$) during the first minute of the post-stimulus period. This was followed by a significant increase in gill ventilation rates within 3–5 min post-stimulus ($P < 0.05$) (Fig. 2).

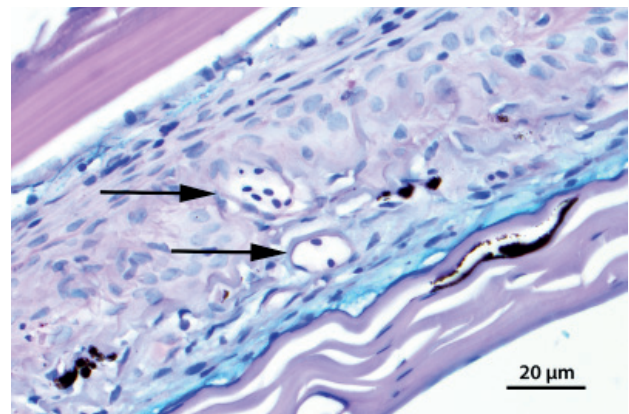


Fig. 1. Transverse section of epidermis of round goby, *Neogobius melanostomus*; Black arrows indicate club cells

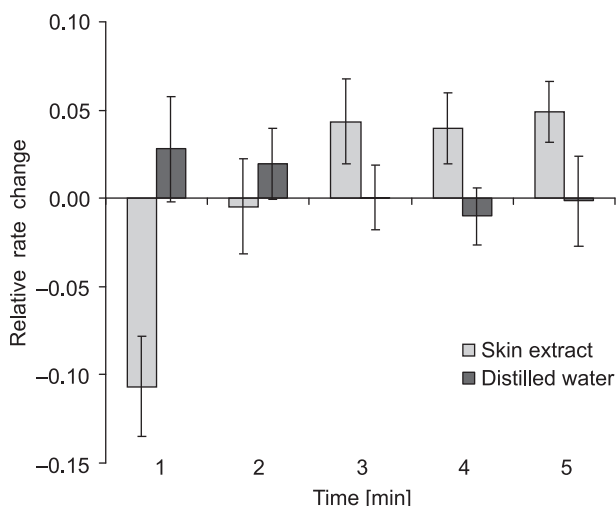


Fig. 2. Relative change in opercular beat rate of round goby, *Neogobius melanostomus*, per 1 min period after exposure to distilled water control and skin extract treatment (values are mean \pm standard error of the mean)

Histological examination of round goby skin revealed the presence of large cells consistent with those of other fish club cells in epidermal tissue. It has been demonstrated that in some fish species alarm cues released following skin damage are associated with epidermal club cells (Smith 1973). These cells originally were described as characteristic of fish in the superorder Ostariophysi (see Pfeiffer 1977), however, they have been subsequently found in many other fish species (Chivers and Smith 1998, Ferrari et al. 2010). Club cells have also been recently identified in the epidermis of gobiid fish (Gobiidae) (Barreto et al. 2014). The primary function of these cells is immune response, and their alarm function evolved secondarily (Wisenden and Smith 1998, Chivers et al. 2007, Halbgehwachs et al. 2009). However, anti-predator responses to injury-released cues are unrelated to club cell function in some species. An esociform (Esociformes) fish, the central mudminnow, *Umbra limi* (Kirtland, 1840), exhibited anti-predator behavioural responses to chemical alarm cues released by damaged epidermis, although the presence or absence of club cells in this species was not considered (Wisenden et al. 2007). Fathead minnows, *Pimephales promelas* Rafinesque, 1820, respond with anti-predator behaviour to skin extract prepared from larval fathead minnows that do not yet possess any epidermal club cells (Carreau-Green et al. 2008). Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), have been reported as having displayed an anti-predator reaction to a conspecific blood cue (Barreto et al. 2013).

Prey can respond to damage-released alarm cues by changing locomotor activity (Smith and Lawrence 1992), timing and duration of shelter occupation (Wisenden et al. 2010), feeding activity (Maniak et al. 2000), social interactions (Barbosa Júnior et al. 2010, Barreto et al. 2010), avoidance of cue labelled areas (Chivers and Smith 1994). Fish also exhibit physiological stress responses to alarm cues (Barcellos et al. 2007). Some fish species have shown

increased ventilation intensity following exposure to predator signals (Gibson and Mathis 2006, Barreto et al. 2010). Response to conspecific injury signals has also been found among some gobies (Gobiidae), expressed as cryptic behaviour—by reduced movement and feeding activity (Smith 1989, Smith and Lawrence 1992, McCormick and Larson 2007, Marsh-Hunkin et al. 2013, Barreto et al. 2014). Decreases in ventilation rate in response to exposure to conspecific skin extract was previously reported for speckled catfish, *Pseudoplatystoma coruscans* (Spix et Agassiz, 1829) (see Barreto et al. 2012). The decrease in gill ventilation is suggestive of cryptic behaviour as an anti-predator reaction and, consequently, helps fish to become less detectable by predators (Mathis and Smith 1993). The magnitude of increase in gill ventilation rate during the later phase was considerably lower, however more prolonged, when compared to the rate of decrease during the first minute after exposure to the skin extract treatment and might be a compensatory process that follows a period of hypoventilation.

The changes in the opercular rate observed in this study cannot be conclusively attributed to an anti-predatory response to the skin extract. Although change in gill ventilation rate has often been suggested to be a response to stress (Barreto and Volpato 2004), it may also be related to feeding behaviour. Skin extract contains a mix of different substances which can induce feeding behaviour in fish as well, and therefore a change in feeding may be responsible for the observed change in the opercular rate. This alternative hypothesis, however, needs further testing via studies on round goby behavioural responses to conspecific damage cues.

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