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Assessment of the use of *Oblada melanura* (L. 1758) otolith fluctuating asymmetry as environmental disturbance indicator

P.S.P. Vandenbussche*, G. Spennato, P.M. Pierson

Université Nice Sophia Antipolis, UCA, CNRS, FRE 3729 ECOMERS, Faculté des Sciences, Parc Valrose, 28 Avenue Valrose, 06108 Nice Cedex 2, France

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A B S T R A C T

Human impact on the environment is of widespread concern. The majority of anthropogenic impacts are centred on coastal ecosystems, so surveying them is an important step in the protection of the marine environment. We have tested *Oblada melanura* (L. 1758) otoliths' fluctuating asymmetry as a bioindicator in a Mediterranean coastal zone. The French Riviera is characterised by a summer population increase leading in particular to more yachting, and seasonal climatic changes with reduced, more concentrated waterway flows and storm events causing soil erosion. The present three-year study compares nine sites, situated in three zones, and characterised by three types of chemical pollutant states (low; waterway mouth; recreational harbour). For *O. melanura* juveniles, we have not shown any significant difference in the otoliths' fluctuating symmetry between zones or types of sites. We hypothesize that high stress levels are needed to induce significant fluctuating asymmetry variation.

1. Introduction

Assessment of human impact on the marine environment is of major interest. Chemical methods are today widely used in environmental surveys because of their reliability that makes them suitable for use by legislators. Despite the advantages of these inert methods, biomonitoring has been developed in order to show impacts on living organisms. In marine environment, some organisms are used as bioindicators, such as plants (Marbà et al., 2012; Papathanasiou et al., 2016), holobionts such as corals (Cooper and Fabricius, 2012), or animals such as invertebrates (Warwick et al., 1990) or vertebrates such as fishes (Cresson et al., 2015). In the present study, we wanted to test a new and easy-to-use indicator for stress in young fishes which are known to be more sensitive to contamination than adults (Azad, 2013). More specifically, we have measured the impact of disturbance in the fishes' environmental medium on the induction of fluctuating asymmetry in otoliths.

As fluctuating asymmetry (FA) is associated with developmental instability (Palmer, 1994; Palmer and Strobeck, 1986, 2003a), it has been shown to be an interesting stress impact indicator for different stressors such as nutritional conditions (Grønkjær and Sand, 2003), chemical contamination (Sopinka et al., 2012) or even living conditions such as high eutrophication level (Almeida et al., 2008). Nevertheless, other studies have shown an absence of relationship between FA and stress (Kruuk et al., 2003; Øxnevad et al., 2002; Vøllestad and Hindar, 2001). Because of publication bias, with positive results preferentially published, the number of 'negative results' is probably underestimated (Debat, 2016; Díaz-Gil et al., 2015; Palmer, 1999).

To study FA, particularly in the case of young individuals, choosing internal structures which are not susceptible to damage by sampling methods is more appropriate and more reliable. Moreover, these internal structures must be solid, such as bones or otoliths. Young juveniles' bones, from our observations, were not accessible without been damaged. Otoliths, which grow daily (Parmentier et al., 2007), are of interest for monitoring environmental change over a period of a few days. In young fishes, daily increments represent a higher proportion of otolith total size than in older fishes (Anken et al., 2002), so their otoliths are more suited to 'recording' environmental information on short-term exposure to stressors than those of adults which are known to respond to long-term exposure (Campana and Casselman, 1993). It has been shown that in young fishes' otoliths, the asymmetry may change rapidly according to environmental conditions (Anken et al., 1998). On this basis, we have focused our study on fish juveniles.

Oblada melanura (Linnaeus 1758) juveniles, which are sedentary, are particularly well-suited for monitoring the environmental influence on development.

The aim of this work was to assess the possible use of *O. melanura* otoliths' fluctuating asymmetry (FA), by means of six morphological parameters (Kristoffersen and Magoulas, 2009), as an indicator of environmental stress, in particular as a chemical pollution indicator. This

* Corresponding author.
E-mail address: vandenbussch@unice.fr (P.S.P. Vandenbussche).

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study was conducted along the coasts of the French Riviera in summer. Summer climatic variations are characterised by periods of drought resulting in reduced, more concentrated flows of waterways, and by occasional storm events which cause soil erosion, resulting in contamination peaks of waterways. Furthermore, this period is associated with more intensive human activities in the sea, such as yachting (Petrosillo et al., 2009). Hence, we have selected nine sites distributed in three adjacent geographical zones. As currents in this area run from east to west, with the gulf of Genoa (a major industrial area) to the east, we were interested to know whether the distance from this gulf to each zone may have an impact. In each zone, three types of sites were chosen to represent three different types of chemical pollution according to the literature. The first type of site presents the lowest possible level of anthropogenic activity, is not affected by waters from waterways or harbours through sea currents and is not close to an identified source of pollution. The second type of site is a waterway outflow into the Mediterranean. It has been demonstrated, in several areas including the French Riviera, that waterways carry mostly trace metals (Du Laing et al., 2009; Fukai et al., 1975; Khan et al., 2014), so carry chemical pollutants to the sea. Finally, the third type is a mid-sized recreational harbour, the French Riviera being a famous yachting area. It is well known that harbours, including those of the French Riviera, are polluted (McCoy and Johnson, 2010; Mestres et al., 2010) mainly due to fuel (Telli Karakoc et al., 1997) and boat paints which contain metals (Damiens et al., 2007; Konstantinou and Albanis, 2004).

2. Materials and methods

2.1. Species of interest

Oblada melanura is a sparid fish spawning in late spring/early summer. Its small juveniles live in shoals, between the surface and 2 m depth, in proximity to cavities which can provide shelters, on hard natural and artificial substrates (e.g. seawalls or jetties). In this study, the mean standard length ± SE is 10.65 ± 0.05 mm (2013: 12.01 ± 0.15; 2014: 10.17 ± 0.04; 2015: 10.45 ± 0.08).

2.2. Sampling design

In 2013, 2014 and 2015, a total of nine sites were sampled. These sites were distributed between three adjacent zones along the French Riviera, with three types of site in each zone. The location of sampling zones is presented in Fig. 1, GPS positions of our sampling sites are presented in Table 1. In the sampling area, sea currents run from east to west, from Zone 3 to Zone 1, which are located at a progressively greater distance from the gulf of Genoa. As stated above, the types of sampling sites have been chosen on the basis of types of chemical pollution. The first type of site, corresponding to the lowest level of pollution, selected according to geographical location, sea current patterns and distance from waterways or harbours in order to be preserved as much as possible from their influence, is referred to as “Type A”. The second type of site, corresponding to a waterway mouth, carrying metals (Du Laing et al., 2009; Fukai et al., 1975; Khan et al., 2014), is not affected by harbours (with regard to distance and sea current patterns) and is referred to as “Type B”. The third type of site, corresponding to a mid-sized recreational harbour, with pollution by fuel (Telli Karakoc et al., 1997) and boat paint containing metals (Damiens et al., 2007; Konstantinou and Albanis, 2004), is not under the impact of waterways (with regard to distance and sea current patterns) and is referred to as “Type C”.

2.3. Sampling procedure

From the 20th of May, a visual survey was conducted twice a week, at the eastern site (Type A of Zone 3) because of the direction of the main currents of the area, in order to identify the first arrival of *Oblada melanura*. Fishes were sampled one week after they were first encountered at selected sites. They were collected between 10/07 and 26/07 in 2013, 23/06 and 01/07 in 2014 and 15/06 and 29/06 in 2015. Sampling in 2013 was later than in 2014 and 2015 because of the late arrival of the fish. In 2015, we did not encounter any *Oblada melanura* at site 2C. A total of 1344 *Oblada melanura* juveniles were collected (Table 2). Sampling was conducted during the morning. Landing nets were used to collect the samples.

Fishes were immediately put in a 2.038 $g\,l^{-1}$ clove oil seawater solution for euthanasia by overdosing (Neiffer and Stamper, 2009). This concentration represents 50 times the anaesthetic dose for sparid fishes such as *Sparus aurata* and *Dentex dentex* (García-Gómez et al., 2002; Ross and Ross, 2008). Fishes were then transported to the laboratory in an icebox cooled with icepacks and frozen at −20 °C for preservation until preparation.

2.4. Preparation

Fishes were measured to the nearest 1/10 mm and weighed to the nearest mg. Otoliths (*sagittae*) were dissected, under a binocular

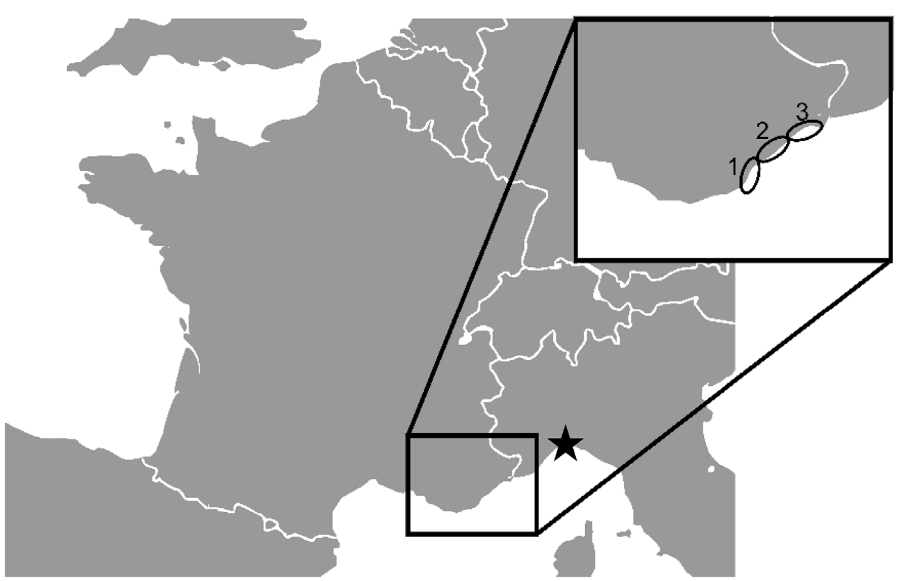

Fig. 1. Map of sampling zones. Ellipses: sampling zones 1, 2, 3; star: Genoa.