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Relationships between growth, survival, physiology and behaviour — A multi-criteria approach to *Haliotis tuberculata* phenotypic traits

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ABSTRACT

Abalone growth rate is often identified among important traits to improve through selective breeding. However, the rapid success of some selective breeding plans has sometimes led to negative effects in some aquaculture species due to trade-offs. One of them is the loss of homeostasis of selected animals which results in the inability to resist the stress experienced during the rearing process. In this context, this study aimed to analyze the phenotypic relationships between growth, and physiological and behavioural traits in *Haliotis tuberculata* under stressful conditions. Eleven traits related to growth, immunity, reproduction and behaviour were recorded under laboratory conditions. A total of 120 adults from wild or farm origin were first monitored during a 3-week stress period (high density and acute stress handling) during winter, followed by 6 months on-growing in sea-cages. Relationships between parameters were analyzed using a multi-factorial approach. Wild and farm stocks could only be discriminated on behaviour traits, confirming that the French abalone industry is in the beginning of the domestication process. After 3 weeks of chronic stress, the righting latency of an abalone was linked to better survival and faster growth. Abalones having the best growth after 6 months were characterized by higher activity during the previous winter period, whereas an early gonad maturation reduced the growth in summer. Our results provide a basis for the establishment of a multi-trait breeding program to improve the growth rate while controlling the evolution of physiological and behavioural traits.
Statement of relevance: The relationships of behavioural and physiological variables with survival and weight gain after application of important stressors were studied in order to provide a better understanding of *Haliotis tuberculata* biology during early domestication stage. This paper will give information on new targets and tools for selective breeding.

1. Introduction

Abalones (*Haliotis sp.*) have been consumed in Asia for centuries as a part of traditional food and are currently consumed in many countries (Elliott, 2000). Worldwide demand has exceeded its supply. This has resulted in an excessive pressure on the stocks in many fisheries (Gordon and Cook, 2004) and a global decrease of the wild stocks (Huchette and Clavier, 2004). The high market value of abalone has allowed an impressive development of aquaculture production throughout the world in the last two decades (Lucas et al., 2006). The success of abalone production is often consolidated by selective breeding plans that generate production gains (Camara and Symonds, 2014; Elliott, 2000).

Haliotis tuberculata is a European species that has been brought into aquaculture in the 2000s. This gastropod is nocturnal and grazes macro-algae found on the rock or drifting algae. It is a slow growing abalone: four years are necessary to reach a commercial size of 7–8 cm (Huchette and Clavier, 2004). The European abalone *Haliotis tuberculata* is a species sensitive to environmental changes (Cenni et al., 2010). *H. tuberculata* is gonochoric and starts its maturation at the end of winter in Brittany and is fully mature in the (Travers, 2008). No genetic improvements have yet been obtained through selective breeding or chromosome manipulations. This European industry would benefit from similar gains to those obtained for other abalone species worldwide (Rhode et al., 2012). However, in many animal production systems, individuals submitted to high productivity improvement seem to be more at risk from behavioural, physiological and immunological

Abbreviations: ANOVA, Analysis of Variance; PCA, Principal Component Analysis; SEM, Standard Error of the Mean.

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issues (Rauw et al., 1998). To avoid that, the current selective breeding strategy is to more carefully define the trait to improve (Camara and Symonds, 2014; Elliott, 2008) and to have a multi-criteria approach, adding physiological, behavioural and metabolic traits to complement production traits (Camara and Symonds, 2014; Rauw et al., 1998). This methodology is used in agricultural production but requires a good biological knowledge and efficient phenotyping methodologies (Monget and Le Bail, 2009).

Domestication is a "long process by which a population of animals becomes dependent on and adapted to humans and to a captive environment by some combination of genetic changes occurring over generations and environmentally induced by events reoccurring in each generation" (Price, 1984). During the domestication process, animals are bred in captivity and encounter several stressors related to captivity environment such as high density or suboptimal conditions. The culture environment reduces the pressure of natural selection to adapt on many traits, but introduces new pressures on other traits (Lorenzen et al., 2012). Abalone is a sensitive species to stressors (Hooper et al., 2011; Travers, 2008; Travers et al., 2008a). High density (Huchette et al., 2003), high ammonia concentration (Cheng et al., 2004a), high temperature and heat shock (Cheng et al., 2004b; Hooper et al., 2014), manipulation (Hooper et al., 2011) and shaking (Malham et al., 2003) are well identified farm stressors that affect the abalone immune system and increase the diseases susceptibility to pathogens. Thus after a period of domestication, captive 'type' abalone might be already more adapted to the culture environment (Lorenzen et al., 2012).

All these previous studies emphasized the importance of linking farm stressors and husbandry practices to animal biology in order to understand growth and mortalities in farms. A biometric study has already been performed on *H. tuberculata* indicating that length, width, weight, meat yield and gonad weight ratio as well as different growth parameters are highly correlated (Basuyaux, 1997). In addition, an immune screening profile was also carried out on *H. tuberculata* (Travers et al., 2008c). For abalone, among many immune parameters, the phagocytosis efficiency (Hooper et al., 2007; Travers et al., 2008b, 2008c) seemed to be a key parameter to predict survival in a rearing system. A diminution of phagocytosis efficiency is often induced by manipulation and environmental condition changes. In addition, study has shown a relation between disease susceptibility and gonadic development (Travers et al., 2008a).

In complement to physiology, behavioural observations are also a useful tool to evaluate an animal's adaptation to an aquaculture rearing system (Claireaux et al., 2015; Millot et al., 2010; Robinson et al., 2013). The righting test, which consists of measuring the time needed for an abalone to fully complete a righting movement after it has been placed upside down was used as a non-invasive tool to provide an evaluation of the energetic reserve of an abalone (Baldwin et al., 2007). Abalone species are generally sedentary, for example *H. iris* spend <6% of their time moving (Allen et al., 2006). *H. tuberculata* is characterized by two patterns of behaviour: "wanderer" abalone move and occupy hides, showing a more pronounced homing behaviour than "sedentary" abalone (Cenni et al., 2009). More movements were also observed during the 24 h following an acute stress (Robinson et al., 2013). In addition, this locomotion is negatively correlated with food availability (Allen et al., 2006).

This study presents a screening of the *Haliotis tuberculata* biology under farm stressors. The first aim was to describe production, physiological and behavioural traits of *H. tuberculata* comparing wild and unselected farm stocks. The second aim was to understand which behavioural and physiological traits were related to growth and survival capacity in stressful conditions, using multivariate analysis.

2. Materials and methods

2.1. Animals

Farmed adult *H. tuberculata* ($n = 60$ abalones in total, 68 ± 4.0 mm total shell length, 3.5 years old) were sampled randomly from 9 sea-based breeding structures of the France Haliotis abalone farm (48°36′46N; 4°33′30W, Plouguerneau, France) in winter. This farm started in 2004. The France Haliotis stocks resulted from systematic mating between wild and farmed broodstock, mainly to avoid inbreeding. Wild adult *H. tuberculata* ($n = 60$ abalones in total, 71 ± 4.0 mm total shell length, age unknown) were also collected the same day by diving in two areas next to the cages, separated from them with at least 100 m of sandy bottom. In addition, farmed stocks could be easily identified with the green coloration at the apex, distinctive of juvenile feed with *Ulvella lens* in France Haliotis hatchery. Both farmed and wild abalones were placed in bags containing circular black plastic oyster seed collectors (diameter: 140 mm) and transported to the land-based laboratory. They were placed in experimental tanks for 3 weeks before transferring them to sea-growing structure for 6 months.

2.2. Experimental set-up

The experimental tanks were 4 grey flat sub-square epoxy painted fibreglass tanks ($1.1 \times 1.1 \times 0.20$ m, water volume $= 100$ L) with rough plastic strips on the edges to prevent escape. Each tank was supplied with 75 $L \cdot h^{-1}$ of 3 μm mechanically filtered seawater (average 10 ± 1 °C) and an aeration system was placed in each tank. Ten pairs of oyster seed collectors were uniformly distributed along the tank edges to be used as hiding places. The light dark regime was adjusted to the seasonal rhythm 10:14 h (Light from 08:30 to 18:30). To avoid stressful conditions during light changes, 30 min dawn (8:00 to 8:30) and dusk (18:30 to 19:00) transitions were regulated using a dimmer (Gold Star, Besser Elektronik, Italia). Tanks were cleaned twice a week using a hose and 25 μm filters changed every day. The algae *Palmaria palmata*, *Saccharina latissima*, *Laminaria digitata* and *Ulva lactuca* were placed *ad libitum* in plastic boxes in the middle of the tank. All tanks were continuously videotaped with 4 digital cameras (TS-WD6001HPSC, Sygonix Gmbh, Germany), linked to a 24 h recording device (TVVR 40021, Abus, Germany). The righting tests were video-recorded for 4 min with a camcorder (Sony, HDR-XR155).

The sea-based growing experiment took place in Allibert. The growing structure was composed of 4 square compartments ($1 \text{ m} * 1.2 \text{ m} * 0.5 \text{ m}$) designed for abalone rearing. Each compartment contained 10 rows of 41 black plastic oyster collectors. Abalones were fed once a month with *Laminaria digitata*, distributed *ad libitum* in the compartment.

2.3. Experimental design

Behavioural and physiological measurements were performed in the laboratory from the end of December 2013 to February 2014 with 3 repetitions. For each repetition, 2 tanks containing farmed abalone and 2 tanks containing wild abalone ($n = 10$ abalone per tank) were used. After gently detaching the abalone with a spatula from the transport collectors, length and weight were measured and abalones were individually marked with a reflective tag and a numbered plastic tag attached with cyanoacrylate glue to their shell (Shepherd and Cannon, 1988) before they were placed in the experimental tank.

Observations were made during three stress periods that were imposed in the experimental tanks, corresponding to three stressors regularly experienced during husbandry procedures:

- Habituation period (day 1 to 7) corresponded to the period of recovery from transport and handling. This is considered a mild stress. Previous observations have shown that the shipping stress and the tagging stress have no impacts after 3 days (Hooper et al., 2011).
- Over-density period (day 8 to 15): 52 additional abalones were added day 8 in order to induce a high-density stress (62 abalones/m^2). The 52 non-experimental abalones were removed day 15.
- Post-acute stress period (day 15 to 21): on day 15, abalones were detached and shaken for 20 min in air, which is a common stressor