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Short communication

Ocean acidification leads to deformations of caudal vein angio-architecture in juvenile threespine stickleback, *Gasterosteus aculeatus* Linnaeus

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One of the consequences of increased CO_2 concentrations in the atmosphere is a decline in oceanic pH, leading to an acidification of the ocean, which represents a severe environmental stressor for fishes. Due to its direct influence on the ion and acid–base budget, it has the potential to influence the development of fishes (Gillooly *et al.* 2002; Pörtner, Langenbuch & Reipschläger 2004; Daufresne, Lengfeller & Sommer 2009; Doney *et al.* 2009). In particular, vulnerable are fish eggs and larvae with limited ion exchange compared with juveniles and adults (Ishimatsu, Hayashi & Kikkawa 2008; Hurst, Fernandez & Mathis 2013). Acidification stress is known to alter behaviour (Jutfelt *et al.* 2013), growth trajectories and morphological traits of the threespine stickleback *Gasterosteus aculeatus* L. (Schade, Clemmesen & Wegner 2014). In the same fish used in Schade *et al.* (2014) that were reared under different ocean acidification scenarios, we discovered alterations of a major axial vessel, the caudal vein (CV), in the tails of several specimens. In general, the tail of teleost fishes displays an uniform pattern of blood vessels (Weinstein 1999; Childs *et al.* 2002). Immediately ventral to the caudal vertebral column run two major axial vessels, the caudal aorta (CA) and the CV. The CA extends caudally to the tip of the tail, and the CV extends from here rostrally to the posterior end of the abdominal cavity. Both vessels are located in the haemal canal, a bony structure formed by ventral extensions from the caudal vertebrae, with the CV immediately ventral to the CA (Fig. 1). This median position protects the vessels from compressions of the contracting myomeres (Satchell 1992). Both axial vessels give rise to intersegmental vessels (ISV), arteries and veins more or less regularly alternating in every second myomere (Steffens, Lomholt & Vogel 1986; Isogai *et al.* 2003).

This vascular pattern is also characteristic for the threespine stickleback a fish distributed in the Holarctic region where it inhabits marine, brackish and freshwaters (Wootton 1984). This small fish is a model species for evolutionary biology, ecology and behaviour (summarized in Wootton 2009) and well known for its phenotypic (Wootton 1984) and its physiological (Allenbach 2011) plasticity.

Here, we investigated 253 laboratory-reared marine threespine sticklebacks used in a previous ocean acidification experiment (North Sea; Schade *et al.* 2014). These fish were reared in sea water aerated with gas mixtures containing ambient CO_2 level of 435 ± 27 µatm (n = 83) or elevated CO_2 level of 1167 ± 176 µatm (end-of-century predictions for ocean pCO_2; n = 170) for 100 days. To determine induction of malformations by other environmental factors, we also

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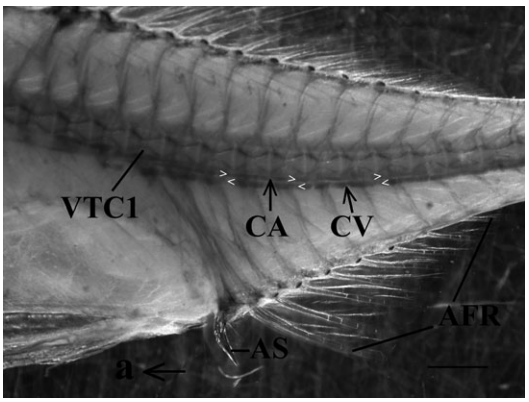

Figure 1 Usual angio-architecture of the CA and CV in *Gasterosteus aculeatus* (lateral view of the caudal region). The CA and CV are visible as the two dark straight lines ventral to the vertebral column. Dark colour of both blood vessels originates from coagulated blood. White arrowheads indicate the direction of the blood flow, caudally in the CA, cranially in the CV. AFR, anal fin rays; AS, anal fin spine; CA, caudal aorta; CV, caudal vein; VTC1, first caudal vertebra; a, anterior. Scale bar = 1 mm.

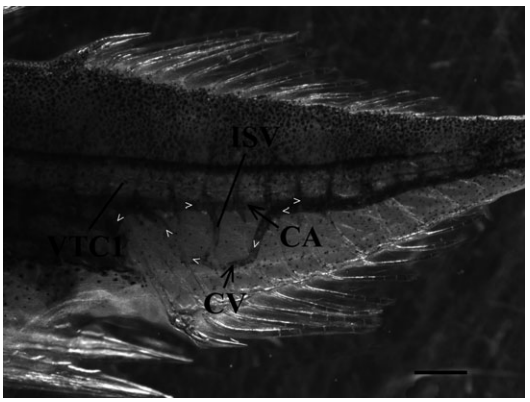

Figure 2 Deformed CV in *Gasterosteus aculeatus* observed in fish from high-CO_2 acidified environments. The course of the vein deviated in an arch ventral to the vertebral column. White arrowheads indicate the direction of the blood flow, caudally in the CA, cranially in the CV. CA, caudal aorta; CV, caudal vein; ISV, intersegmental vessel; VTC1, first caudal vertebra. Scale bar = 1 mm.

investigated 375 juvenile specimens from the same location reared in sea water at 13, 17 or 21 °C (Ramler *et al.* 2014). Additionally, to estimate natural background levels of malformations, we also screened 67 adult specimens from the wild, 18 from the Sylt-Rømø Bight and 49 from a brook in eastern Austria. The primary intention was to investigate bony structures of these sticklebacks. For this purpose, the fish were made translucent and stained with Alizarin Red S (Pothoff 1984). Due to pigmentation, the course of many blood vessels of the tail, especially the course of the CA and CV, were recognizable by light transmission microscopy (Figs 2 & 3). Typically, the CV extends parallel and ventral to the CA from the first to the last caudal vertebra (urostyle). In all wild adult specimens and in all juvenile specimens from the temperature rearing experiment, the pattern of the large blood vessels followed this typical for teleost fishes (Fig. 1). This was also the case in the control juveniles from the acidification experiment reared in sea water with ambient CO_2 level. Contrary, in 5.3% (logistic regression, deviance CO_2 = 6.08, df = 1, P = 0.008) of the juveniles from several families (logistic regression, deviance Family = 16.89, df = 14, P = 0.262) reared in sea water with elevated CO_2 level, parts of the CV (16.4–57%) were not developed (Figs 2 & 3), indicating an environmental induction rather than a genetic predisposure for this deformation. The missing parts of the CV were substituted by ISV with distinctly enlarged diameter and allowed a detour of the venous blood cranially. The collateral parts of these modified ISV run in the midline of the tail ventral or dorsal to the haemal and neural processes, respectively (Figs 2 & 3). These detours increased the distance blood had to flow by 16.6% on average (range 3–37%), and in the most extreme case, the blood had to overcome six sharp bends (five in an angle of about 90°) strongly increasing the mechanical sheer in the flowing blood (Fig. 3b). Additionally, as the collateral parts of this detoured vessel are likely exposed to mechanical stress exerted by contracting myomeres, we expect less efficient transport of blood, which could have severe consequences on organismal performance.

The loss of parts of the CV and detour of the venous blood was restricted to the anterior two-thirds of the tail of the investigated sticklebacks (Figs 2 & 3). In the last third of the tail (distance posterior to the anal fin), the CV was present and showed no malformations in all specimens. Several mutations cause malformations in the pattering of blood vessels during embryogenesis (Weinstein 2002; Jin *et al.* 2007; Baldessari & Mione 2008). In mutant zebrafish embryos, collateral vessels, formed by interconnections of ISV, enable blood flow to bypass malformations (Weinstein *et al.*

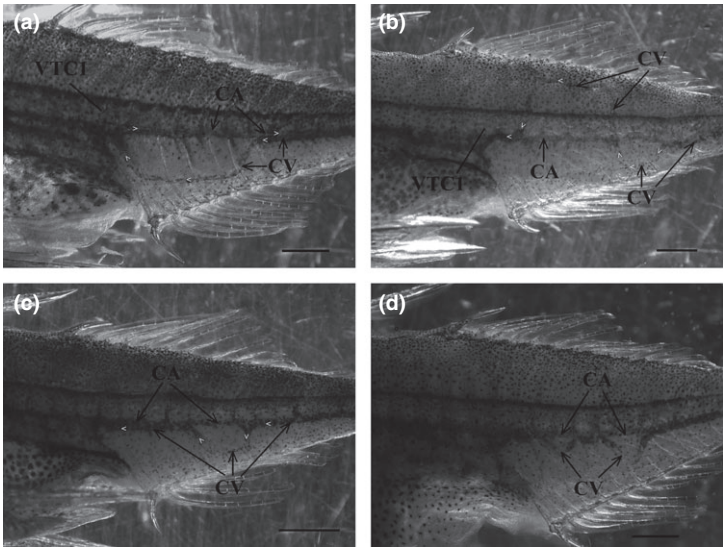

Figure 3 Different examples of deformed CVs in *Gasterosteus aculeatus* (lateral view of the caudal region). White arrowheads indicate the direction of the blood flow, caudally in the CA, cranially in the CV. (a) one large arc ventral to the vertebral column. (b) one arc ventral and a second arc dorsal to the vertebral column. (c) one arc ventral to the vertebral column. (d) two small arcs ventral to the vertebral column. CA, caudal aorta; CV, caudal vein; VTC1, first caudal vertebra. Scale bar = 1 mm.

1995; Hogan *et al.* 2009). Such vessels have also been described for the grass goby *Zosterisessor ophiocephalus* (Pallas; Lahnsteiner, Lametschwandtner & Patzner 1990). Nevertheless, in all these specimens, collateral ISV occurred in addition to a normally and completely developed CA and CV.

We are not aware of environmental inductions of incomplete CV in combination with drastic detours of the venous blood in post-larval stages of other fish species like demonstrated here for threespine sticklebacks. Obviously, the affected specimens were not only able to compensate the loss of parts of the CV but also to reach development distinctly beyond the larval period generally completed after about 22 days post-hatching and at a size of about 11 mm (Swarup 1958). The smallest of our investigated specimens with a deformed CV had a size of 22 mm SL, the largest a size of 29 mm SL. Contrary to the CV, the CA of all investigated specimens including those reared in sea water with elevated CO_2 level showed no malformations. The formation of the major axial vessels is differently regulated during embryogenesis (Roman & Weinstein 2000; Ellertsdóttir *et al.* 2010; Wiley *et al.* 2011) and possibly makes the CV more vulnerable to environmental stress due to elevated CO_2 than the CA.

Although recent studies focused on the response of the early development of marine fishes to ocean acidification (Munday *et al.* 2011; Forsgren *et al.* 2013; Pimentel *et al.* 2014; Schade *et al.* 2014), it is not known whether and how an elevated CO_2 level influences the development of the cardiovascular system. The formation of the vascular pattern of fishes is not solely based on a genetic program but also responds to environmental changes (reviewed in Pelster 2003).

While it is true that on average a more gradual change in pCO_2 concentration can be expected in natural systems, some ecosystems already experience similarly steep shifts in pCO_2 concentrations today (Melzner *et al.* 2013). If such pCO_2 surges coincide with critical developmental periods, deformations as observed here may also occur in natural populations. However, if organisms experience a more gradual change of environmental conditions, they might be able to adapt, and ultimately, the gradients of environmental change will determine the developmental consequences of ocean acidification in marine fish populations.

If increasing ocean acidification has the potential to overcome developmental constraints of the formation of blood vessels, this could have severe effects on the early life history of marine fishes. Further studies should investigate the occurrence and effects of deformed cardiovascular systems on organismal performance to assess the consequences of ocean acidification more precisely.

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