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Influence of crude oil exposure on cardiac function and thermal tolerance of juvenile rainbow trout and European sea bass

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Abstract :

Oil spills pose a threat to aquatic organisms. However, the physiological effects of crude oil on cardiac function and on thermal tolerance of juvenile fish are still poorly understood. Consequently, in this paper, we will present results of two separate experiments where we exposed juvenile rainbow trout and European sea bass to crude oil and made cardiac thermal tolerances and maximum heart rate (fHmax) measurements after 1 week (rainbow trout) and 6-month recovery (sea bass). In both species, the fHmax was lower in crude oil-exposed fish than in the control ones at temperatures below the optimum but this difference disappeared at higher temperatures. More importantly, the oil-exposed fish had significantly higher Arrhenius break point temperature for fHmax, which gave an estimate for optimum temperature, than the control fish in both species even though the exposure conditions and recovery times differed between species. The results indicated that exposure of juvenile fish to crude oil did not have a significant negative impact upon their cardiac performance in high temperatures and upper thermal tolerance increased when the fish were tested 1 week or 6 months after the exposure. Our findings suggest that the cardiac function and thermal tolerance of juvenile fish are relatively resistant to a crude oil exposure.

Keywords : Arrhenius break point temperature, Critical thermal maximum, CTMAX, Fish, Heart rate, Oil spill, PAH

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Introduction

Accidental oil spills and crude oil exposure represent a threat to aquatic environments worldwide, affecting not only aquatic animals but also human activities (e.g. fisheries, aquaculture and tourism) (Endersen et al. 2003; Meski and Kaitaranta 2014). Previous studies have shown that fish embryos and larvae are particularly sensitive to crude oil and its components, especially polycyclic aromatic hydrocarbons (PAH). The heart is a particularly sensitive organ, with numerous reports associating crude oil exposure with malformations such as reduced cardiac looping and pericardial edema in developing fish (Thomaz et al. 2009; Incardona et al. 2009, 2012, 2014; Jung et al. 2013). Cardiac dysfunctions such as reduced ventricular contractility (Jung et al. 2013), increased occurrence of arrhythmias and variability of heart rate have also been reported in embryos (Incardona et al. 2009, 2012, 2014, Jung et al. 2013; Sørhus et al. 2016; Khursigara et al. 2017). Furthermore, it has been shown that a year after embryonic stages have been exposed to petroleum hydrocarbon, young fish still exhibit misshaped hearts and lower critical swimming velocities (U_{CRIT}) than unexposed control fish (Hicken et al. 2011).

When comparing embryos to juveniles or adult fish, it seems that juveniles and adults might be less dramatically affected by exposure to petroleum hydrocarbons than younger life stages, although a large interspecific variability has been reported (e.g. Vosyliene et al. 2005; Davoodi and Claireaux 2007; Claireaux and Davoodi 2010; Milinkovitch et al. 2012; Claireaux et al. 2013). For example, common sole (*Solea solea*) and adult mahi-mahi (*Coryphaena hippurus*) showed reduced cardiac output when measured shortly after an exposure to crude oil (Davoodi and Claireaux 2007; Nelson et al. 2016). Johansen and Esbaugh (2017) observed that a 24h acute exposure to 4.1 μg L^{-1} ΣPAH reduced U_{CRIT} and

burst swimming capacity of adult red drum (*Sciaenops ocellatus*) while there was no change in aerobic scope, cost of transport and in the capacity to repay oxygen debt following exhaustive exercise. Brette et al. (2014) exposed isolated cardiomyocytes of juvenile bluefin tuna (*Thunnus orientalis*) and yellowfin tuna (*Thunnus albacares*) to dilutions (20%, 10% and 5%) of high-energy water-soluble fraction (WAF) of oil samples collected from the *Deepwater Horizon* spill. These authors observed impaired cardiomyocyte function during the exposure, with reduced amplitude and tail current of the delayed rectifier potassium current, resulting in prolonged action potential duration (Brette et al. 2014). It must be noted, however, that the lack of effects has also been reported in the literature. For instance, aerobic scope, basal and active metabolic rates as well as U_{CRIT} were unchanged in juvenile golden grey mullet (*Liza aurata*) 24h after oil exposure (Milinkovitch et al. 2012). Claireaux et al. (2013) showed that 1 week after an exposure to crude oil or to chemically dispersed oil, juvenile European seabass (*Dicentrarchus labrax*) displayed reduced hypoxia tolerance (incipient lethal oxygen saturation) and thermal sensitivity (critical thermal maximum, CT_{MAX}) compared to unexposed, control fish. These differences were, however, no longer observed 4 weeks (Claireaux et al. 2013) or 10 months post-exposure (Mauduit et al. 2016).

Besides crude oil, fish are also exposed to other environmental changes in nature. One of these is the ongoing climate change. Anthropogenic activities are changing the earth's climate and water temperature has progressively increased around the world. This increase is directly affecting the physiology of ectothermic fish and it is expected to have detrimental effects if populations cannot migrate to new areas or increase their thermal tolerance via phenotypic plasticity or adaptation through evolution by natural selection (Parmesan 2006; BACC Author team 2008; Belkin 2009; Pörtner 2010; Marshall et al. 2014). The question is, thus, to examine whether exposure to petroleum hydrocarbon compounds affects the capacity

of fish to face warming events, such as heat waves for instance, the occurrence of which is predicted to increase with climate change (e.g. Teng et al. 2016). In this paper, we investigated the general nature of the consequences of crude oil-exposure on cardiac performance and thermal tolerance of juvenile fish. In these experiments, juveniles of two ecologically different fish species, the freshwater rainbow trout (*Oncorhynchus mykiss*) and the seawater seabass were exposed to crude oil of different origin (trout: Russian export blend medium crude oil; seabass: Arabian light crude oil), with (seabass) or without (trout) treatment by dispersant. These species were chosen since they are important fisheries/aquaculture species and are living/reared in areas where the threat from oil accident is especially high (BACC Author team 2008; Marshall et al. 2014). Rainbow trout were studied 1 week post exposure while seabass were examined 6 months post exposure. The hypothesis was that oil exposure reduces the CT_{MAX} and cardiac function of both species and that the effects persist even after the fish have recovered in clean water.

Materials and Methods

This study contains two separate experiments that were done independently from each other, one in Finland with rainbow trout and one in France with seabass. In both experiments the endpoint measurements i.e. CT_{MAX} and maximum heart rate were done similarly and the results were remarkably consistent. Therefore, these separate experiments are shown together as they give a uniform general view of how the crude oil exposure influences cardiac performance and thermal tolerance in juvenile fish. The measuring techniques (i.e. CT_{MAX} and heart rate recordings) were used as screening tools and both techniques provided separate estimates of upper thermal tolerance of fish even though, in both cases, the rate of increase of temperature was higher than in natural situations.

Since the experiments were done independently there were some differences between experiments that need to be noted. First, the origin of the crude oil used was different between experiments. Russian Export Blend medium crude oil was used in the rainbow trout study and Arabian light crude oil in the seabass study. Seabass were exposed to a ten times higher oil concentration than rainbow trout, since it had previously been shown that a lower concentration did not have a significant long-term effect on the thermal tolerance of seabass (Claireaux et al. 2013; Mauduit et al. 2016). Further, in seabass experiment chemical dispersant and weathering was used while in the rainbow trout study the crude oil was weathered. Water Total Petroleum Hydrocarbons (TPH) was analyzed in both experiments whereas PAHs were analyzed in the water in the trout experiment and in the liver in the seabass experiment.

Experiment 1. Rainbow trout

The experiments with rainbow trout (age 1+) were conducted at University of Turku, Finland during summer 2014. The experiments were approved by Finnish Animal Experiment Board (ESAVI/4068/04.10.07/2013). The rainbow trout were obtained from a nearby commercial fish farm (The College of Fisheries and Environment, Kirjala, Finland) and the fish were transported to University of Turku 4 weeks before experiments. Fish were maintained under natural photoperiod and temperature (16°C) conditions in their acclimation tanks (500 L). Fish were fed daily with commercial pellets (Raisio Group, Raisio, Finland) and the feeding was ceased 24h before any experiments/manipulations. The mean size of fish did not differ significantly between experimental groups and the weights and lengths were 4.6±0.5 g and 8.2±0.2 cm (fork length) for control rainbow trout, 4.4±0.3 g and 8.1±0.1 cm for oil-exposed rainbow trout.

Exposures

Russian Export Blend medium crude oil was acquired from Neste Oil (Raisio, Finland) and weathered at 22°C by bubbling air through the oil layer until approximately 10% of the oil mass was lost. Such a treatment of the oil simulated a 12h aging of a slick released at sea (Nordvik 1995). Fish exposures were conducted according to Milinkovitch et al. (2011). Fish were allocated to two subgroups (oil-exposed and control, n = 60 fish per group) and transferred from their acclimation tanks to identical, polyethylene tanks (185 L; 3 tanks per treatment) 48h before exposures (biomass per tank 1.46 g L^{-1}). The exposure tanks were equipped with air stones and custom-made mixing system, which allowed full homogenization of water column and kept oxygen level above 80% of air saturation throughout the exposures (see Milinkovitch et al. 2011 for further details about mixing system). Temperature of exposure tanks was kept at fish acclimation temperature (16°C). The exposure was started by pouring 12.5 g of weathered crude oil to the surface of the tanks (i.e. the oil concentration was 0.07 g L^{-1}) and lasted 48h. One liter water samples were taken from the middle of the water column of each tank, at the beginning and at the end of the exposure period. The TPH and PAH concentrations were analyzed from the water samples by Novalab Oy (Karkkila, Finland). Chemical analyses protocols are described in the supplementary materials. The PAHs could not be measured from tissue samples in Novalab Oy. Following exposure rainbow trout were briefly bathed in clean water containing 70 ppm buffered MS-222 and the adipose fin cut to identify the fish later on. After bathing, fish were returned to their initial acclimation tanks so that both control and exposed fish were under garden conditions thereafter. Control fish followed the same protocol except that no chemicals were added to their tank. No mortalities were observed during the exposure and the following week.

The pH of the water did not change during the exposure (7.5±0.1) and nitrogen waste concentrations stayed below detection limits (nitrite <0.25 mg L^{-1}, nitrate <10 mg L^{-1} and ammonium <0.5 mg L^{-1}). The oxygen concentration and water temperatures were 9.2±0.1 mg L^{-1} and 16.3±0.05°C, respectively.

CT_{MAX} experiments

The first aim of our study was to examine whether a crude oil exposure, followed by a significant recovery period in a clean environment, influenced the upper critical thermal tolerance (CT_{MAX}) of fish. This was verified by exposing control and oil-treated fish to an acutely increasing temperature until the fish could no longer keep an upright position. The value of CT_{MAX} indicated the upper thermal tolerance of a fish beyond which survival was time-limited (Sunday et al. 2015).

The CT_{MAX} experiments with rainbow trout were conducted 7 days after the oil exposure according to Anttila et al. (2013b). Briefly, both control and exposed rainbow trout (n = 15 per group) were transferred to the experimental tank (100 L, 16°C) 1h before the experiment started. At the end of the acclimation period, the temperature of the water was increased at a constant rate of 0.3°C min^{-1} up to 24°C and by 0.1°C min^{-1} thereafter until the fish lost equilibrium. Water temperature was controlled with a circulating 2500 W heater (RC6, Lauda, Lauda-Königshofen, Germany). Water oxygenation and homogeneity were assured by bubbling air vigorously into the tanks and using aquarium pumps to circulate the water and keeping the oxygenation level above 80% of air saturation throughout the experiments. Once a fish lost equilibrium, the water temperature was recorded and the fish was quickly removed from the tank and placed in a recovery tank (all the fish had their own 10 L recovery tanks) at

the acclimation temperature. After the experiment, the rainbow trout were euthanized with 200 ppm MS-222 buffered with sodium bicarbonate. The mass and length of the fish were recorded.

Heart rate measurements

The second aim of the study was to measure whether a crude oil exposure influenced the maximum heart rate (f_{Hmax}) of the fish at different temperatures. With this technique we were also able to estimate the upper thermal tolerance of cardiac function. The temperature where f_{Hmax} stops increasing exponentially with increasing temperature (Arrhenius break point temperature, T_{AB}) has been connected to optimum temperature of aerobic scope and growth in several species (rainbow trout: Anttila et al. 2013a; coho salmon, *Oncorhynchus kisutch*: Casselman et al. 2012; Atlantic salmon, *Salmo salar*: Anttila et al. 2014; sockeye salmon, *O. nerka*: Chen et al. 2013; chinook salmon, *O. tshawytscha*: Muñoz et al. 2014; Arctic cod, *Boreogadus saida*: Drost et al. 2014 and goldfish, *Carassius auratus*: Ferreira et al. 2014). Thus, T_{AB} was calculated and used as a proxy for the optimum temperature of fish (T_{OPT}). We also measured the temperature (T_{PEAK}) where the highest heart rate (f_{Hpeak}) was found and the temperature where cardiac arrhythmias were observed (T_{ARR}). These temperature values were indicative the upper thermal limit for cardiac function (Casselman et al. 2012; Anttila et al. 2013a).

f_{Hmax} of rainbow trout was measured a week after the exposure (n = 12 per group, different fish from those in CT_{MAX} measurements). The measurements were done according to Casselman et al. (2012) with slight adjustments in drug concentrations. Briefly, the fish were anesthetized in buffered 80 ppm MS-222, weighted and placed in the experimental setup. The set-up consisted of 4 chambers made of a PVC-pipe with sealed ends and top removed so that

fish were completely submerged. A water flow to these chambers was achieved via a chilling/heating unit (RC6, Lauda, Lauda-Königshofen, Germany). Note that a portion of that water flow was diverted, via pipe-mouthpieces, through fish mouth and gills. This water contained a maintenance dose of anesthetics (70 ppm of buffered MS-222). To detect fish electrocardiogram (ECG), two silver electrodes were placed on the bottom of each chambers underneath the fish. The signal from these electrodes was amplified and filtered using a Grass P122 AC/DC Strain Gage Amplifier (Grass Technologies, Warwick, U.S.A.). ECG recording and analysis were performed with BIOPAC data acquisition unit (MP100) and Acknowledge software (ver. 3.9.1.).

Following their transfer into the experimental chambers, fish were equilibrated at the initial temperature (12°C) for 1h, after which they were given an intraperitoneal injection of atropine sulphate (1.8 mg kg^{-1}, Sigma-Aldrich Chemie Gmbh, Munich, Germany) to completely block the vagal tone. Fifteen minutes later fish were given an isoproterenol injection (4 µg kg^{-1}, Sigma-Aldrich Chemie Gmbh, Munich, Germany) to maximally stimulate cardiac β_1-adrenoceptors. Both drugs were dissolved in saline (0.9% NaCl). These drug concentrations had been tested in preliminary experiments to ensure that maximum heart rate was recorded. Once heart rate had stabilized (15 min after the isoproterenol injection), water temperature was increased in 1°C increments at a rate of 10°C h^{-1}. After every temperature increment, heart rate was allowed to stabilize before recording a value. Warming continued until cardiac arrhythmias developed, after which fish were rapidly removed from the apparatus, euthanized by cranial percussion and their weight and length measured.

For each individual fish temperatures corresponding to T_{ARR} and T_{PEAKT} were recorded and the T_{AB} was calculated with Arrhenius plots according to Yeager and Ultsch (1989).

Experiment 2. Seabass

The seabass (age 1+; 11.3 ±1.1 cm; 17.7 ±5.0 g) were obtained from Aquastream Lorient, France and transferred to a 2000-L rearing tank at Unité de Physiologie Fonctionnelle des Organismes Marins, Ifremer, France. They were maintained under natural photoperiod and temperature conditions (10°C at the time of arrival) and fed daily with commercial pellets (Le Gouessant, Saint-Martin de Valgalgues, France). Fish were acclimated 8 weeks to the laboratory conditions before experiments began and feeding was ceased 24h before any experiments/manipulations. Two weeks before experiments started, seabass were anaesthetized (MS-222; 100 mg L^{-1}, Sigma-Aldrich Chimie Gmbh, Munich, Germany) and individually implanted subcutaneously with an identification tag (RFID; Biolog-id, Bernay, France). All the experiments were approved by Ministère Délégué à l'Enseignement Supérieur et à la Recherche.

Exposures

The exposures were conducted as in experiment 1, apart from a different crude oil (Arabian light crude oil) and concentration (0.8 g L^{-1}). A higher concentration was used because it has been previously shown that a lower concentration (0.07 g L^{-1}) did not influence the thermal tolerance of seabass (Claireaux et al. 2013; Mauduit et al. 2016). Furthermore, in the experiment with seabass, crude oil was treated with dispersant (Finasol OSR 52, 0.017 g L^{-1}) while in the experiment with rainbow trout weathered oil was only mechanically dispersed in water. In seabass experiment 250g of crude oil and 10g of dispersant was mixed vigorously in three separate bottles. The oil-dispersant mixture was directly poured in the three 300 L experimental tanks and then bubbled with air overnight to mimic a 12-h aging of an oil slick at sea (Nordvik 1995). The number of exposed seabass per treatment was 180. Exposure

started when sixty fish were transferred for 48h into triplicate exposure tanks that contained only sea water (control group) or dispersant-treated oil (oil group; total biomass per tank was 4.2 g L^{-1}). The pH of the water did not change during the exposure (8.1±0.1) and the oxygen concentration was maintained above 80% of air saturation. Water samples were taken from each tank shortly after the exposure started and at the end of the exposure to analyze the TPH. The water samples were taken from the middle of the water column using a bottle and the TPH in water samples were analyzed at CEDRE (Brest, France). Nine seabass were sacrificed 24h after the exposure in order to measure liver PAH concentrations. Measurements were conducted in CEDRE (see Supplementary material for the methods of chemical analyses).

CT_{MAX} experiments

Experiments were conducted 4 weeks post oil-exposure. The CT_{MAX} protocol (n = 20 per group) differed slightly from the protocol for rainbow trout in that the thermal challenge test was conducted directly in the fish rearing tank. Moreover, temperature was increased from acclimation temperature to 27°C in 2.5 hours and thereafter the heating rate was lowered to 0.5°C h^{-1} until the last fish lost its equilibrium and was removed from the tank (Claireaux et al. 2013). The water temperature was controlled with JULABO F10, 2500 W heater (Seelbach, Germany). Each time a fish lost its ability to maintain an upright position, the water temperature was recorded, the fish PIT tag number was read and it was transferred to a recovery tank for 2h before being returned to its original rearing tank. The percentage of mortality after the CT_{MAX} experiment was below 1%. Fish were then reared under natural photoperiod and temperature conditions until heart rate measurements (6 months later). There were no differences in mortality between groups.

Heart rate measurements

Maximum heart rate of seabass (n = 18 per group, the same fish as in CT_{MAX} measurements) was measured 6 months after oil exposure (water temperature at that time was 17°C). At the time of heart rate measurements, the mean size of fish did not differ significantly between groups (66.4±4.1 g and 17.2±0.3 cm for control seabass and 71±3.7 g and 17.5±0.3 cm for oil-exposed seabass, respectively). The experimental protocol was exactly the same as with rainbow trout except that anesthesia solution did not contain bicarbonate because of the buffering capacity of seawater. Salinity level of the water was 32-33 ppt. The water temperature was controlled with a recirculating chiller/heater (F10, JULABO, Seelbach, Germany) and the ECG was recorded and analyzed with BioPac MP36R (BIOPAC Systems Inc, Essen, Germany) which had an in-built amplification system. The starting temperature of the measurements for seabass was 17°C. The concentration of atropine sulphate was 3 mg kg^{-1} (Sigma-Aldrich Chemie Gmbh, Munich, Germany) and the concentration of isoproterenol was 3.2 µg kg^{-1} (Sigma-Aldrich Chemie Gmbh, Munich, Germany).

Statistical analyses of both experiments

Data normality and homogeneity were tested with Kolmogorov-Smirnov and Levene tests, respectively. Two-way repeated measures ANOVA was used to calculate the differences in f_{Hmax} values between oil-exposed and control fish during warming (temperature and treatment as factors) and was followed by Holm-Sidak *post hoc* test. Both species were tested separately because of differences in starting temperatures and treatments. Two-way repeated measures ANOVAs were also used to analyze 1) the differences in f_{Hpeak} and scope for heart rate (difference between lowest f_{Hmax} and f_{Hpeak}) between exposed and control animals and 2) the differences in T_{AB}, T_{PEAK}, T_{ARR}, CT_{MAX} between exposed and control animals (exposure and measurement as factors). However, since the individuals for CT_{MAX} experiment were

different from individuals used in heart rate experiment for rainbow trout, the differences in CT_{MAX} between exposed and control rainbow trout were analyzed with t-test. All the statistical analyses were performed with SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA). Statistical significance for comparisons of mean values was set at α=0.05. The values are presented as means ± SEM.

Results

Experiment 1. Rainbow trout

Rainbow trout were exposed to 0.07 g L^{-1} of weathered Russian export blend medium crude oil. The mean ∑hydrocarbons in water samples from the three exposure tanks at the end of the exposure period was 3.8±1.1 mg L^{-1} while the sum of 16 US-EPA PAHs was 4.5±0.14 µg L^{-1}. However, it needs to be noted that only naphthalene, acenaphtalene, fluorene and phenantrene concentrations were above detection limits. The list of assayed PAH compounds is shown in a Supplementary Table. There were no mortalities after exposures.

Heart rate

Exposure to crude oil reduced the heart rate at temperatures below and near optimum temperature (12-18°C, optimum around 17°C, Anttila et al. 2013a) ($F_{1,6}$=6.3, P=0.013, Fig. 1a) while there were no differences between oil-exposed and control fish at temperatures >18°C. Oil exposure did not affect the highest f_{Hmax} recorded (f_{Hpeak}) (108 ±5.1 bpm and 116 ±6.7 bpm for control and oil-exposed rainbow trout, respectively; P=0.29). The difference between the lowest recorded f_{Hmax} and the highest recorded f_{Hmax} (scope for heart rate) were 50.2±4.1 and 64.5±6.5 bpm in control and oil-exposed rainbow trout, respectively, and were not statistically different from each other (P=0.088).

Indices of upper thermal tolerance

Oil exposure had a significant influence on rainbow trout thermal tolerance (Fig. 2a). One week post exposure, T_{AB} was significantly higher in oil-exposed (16.3±0.4°C) than in control (15.1±0.4°C) fish (P=0.036, Fig. 1a, 2a). Similarly, all the indices of upper thermal tolerances (i.e. T_{PEAK}, T_{ARR} and CT_{MAX}) were higher in oil-exposed than in control rainbow trout: the differences between oil-exposed and control fish were 1.8°C, 2.3°C and 0.8°C, for T_{PEAK}, T_{ARR} and CT_{MAX}, respectively (Fig. 2a) (T_{PEAK}: P=0.002; T_{ARR}: P=0.033; CT_{MAX}: P=0.005). CT_{MAX}, T_{ARR}, T_{PEAK} and T_{AB} differed significantly from each other (P<0.001).

Experiment 2. Seabass

The ∑hydrocarbons experienced by seabass at the end of the exposure to 0.8 g L^{-1} of weathered Arabian light crude oil mixed with 0.017 g L^{-1} of dispersant (Finasol OSR 52) was 141±7.8 mg L^{-1}. Twenty four hours post-exposure, the sum of 16 US-EPA PAHs and 5 supplementary PAHs (benzo[b]thiophene, biphenyl, dibenzothiophene, benzo[e]pyrene and perylene) in seabass liver (n = 9) was 34 299 ± 8 926 ng g^{-1} . The concentrations of PAHs are detailed in Supplementary table.

Heart rate

The exposure to crude oil caused similar effects in seabass as observed in rainbow trout although experimental designs and post-exposure recovery times were different. For example, in seabass, as in rainbow trout, the oil-exposed fish had lower f_{Hmax} values ($F_{1,5}$=4.8, P=0.03) than the control fish when measured below the optimum temperature (17-22°C, optimum around 22-24°C, Claireaux and Lagardère 1999), (Fig. 1b). However, there were no differences between oil-exposed and control seabass at temperature > 24°C. As in rainbow

trout the oil exposure did not influence the f_{Hpeak} values (P=0.91). The values for f_{Hpeak} were 125±6.7 bpm and 126±4.9 bpm for control and oil-exposed seabass, respectively. The scopes for f_{Hmax} were 34.0±5.0 and 35.7±5.8 for control and oil-exposed seabass, respectively, and the groups did not differ from each other (P=0.83).

Indices of upper thermal tolerance

Again, as in rainbow trout, the oil-exposed seabass had higher T_{AB} (1.0°C difference) compared to control seabass (P=0.05). The T_{AB} values were 19.3±0.3°C and 20.3±0.3°C for control and oil-exposed seabass, respectively (Fig. 1d, 2b). Furthermore, the T_{PEAK} was higher (P=0.03, difference 1.3°C, Fig. 2b) in oil-exposed seabass than in the control. The T_{PEAK} values were 21.8±0.4°C and 23.1±0.4°C for control and oil-exposed seabass, respectively. However, in seabass the T_{ARR} and CT_{MAX} did not differ statistically between oil-exposed and control fish after a 6-month recovery (T_{ARR}: P=0.62; CT_{MAX}: P=0.94, Fig. 2b).

Discussion

Recent studies have demonstrated that embryonic fish exposed to hydrocarbon compounds can display various cardiac malformations and functional disorders (e.g. Thomaz et al. 2009; Incardona et al. 2009, 2012, 2014; Jung et al. 2013; Sørhus et al. 2016; Khursigara et al. 2017). However, there is very limited literature available about the influence of petroleum hydrocarbon exposure on later life stages. Moreover, when available, reports about disturbed homeostasis or functional disorders generally concern a very short period post exposure, preventing attempts to draw the distinction between normal regulatory processes, a stress response or a true, potentially long-lasting, functional disorders. The aim of the current study was, therefore, to measure, in experiments involving two different fish species, how exposure

to crude oil, followed by a significant recovery period in a clean environment, influenced the upper critical thermal tolerance and cardiac function of juvenile fish. We found that a week (trout) to months (seabass) post exposure, crude oil-exposed fish displayed higher (trout) or unchanged (seabass) upper critical thermal tolerance than control, unexposed fish. We also found that crude oil did not have a significant chronic residual influence on the cardiac performance of our juvenile fish at high temperatures. In contrast to what was expected, our estimate of optimal temperature for cardiac function even increased after exposure in both species.

The upper critical temperature (CT_{MAX}) corresponds to temperature at which a fish exposed to a progressive and controlled increase in water temperature is no longer able to maintain an upright position. When the CT_{MAX} is reached survival is time-limited and, thus, CT_{MAX} is used as a proxy for upper critical thermal tolerance of fish (Sunday et al. 2015). In the current study, we observed that the oil-exposed rainbow trout had a significantly higher CT_{MAX} than control fish. In seabass, on the other hand, no difference in CT_{MAX} was observed between control and oil-exposed fish. These values (29.0±0.2°C for rainbow trout acclimated at 16°C and 31.3±0.3°C for seabass acclimated to 17°C) were similar to those reported earlier (29.4°C and 28-35°C, Beitinger et al. 2000; Claireaux et al. 2013). It has previously been found in juvenile seabass that exposure to crude oil (0.07 g L^{-1}) did not affect their upper critical thermal tolerance (CT_{MAX}) when the measurements were done ten months after exposure (Mauduit et al. 2016). The present study, using 12.5 times higher oil concentration, confirms that exposure to petroleum hydrocarbons has no significant, long-term effect on upper critical thermal tolerance in this species. Since we did not measure CT_{MAX} immediately after oil exposure it is not certain whether this lack of effect results from a recovery process or that oil-exposure has simply no effect on thermal tolerance in juvenile fish. However,

Claireaux et al. (2013) have shown that shortly after oil exposure juvenile seabass do display a reduction in CT_{MAX}. This therefore suggests that juvenile fish have the capacity to recover from the initial impact of oil exposure on their upper critical thermal tolerance.

Optimal temperatures (T_{OPT}) of rainbow trout and seabass are 16.5-17°C and 22-24°C, respectively (Jobling 1981; Claireaux and Lagardère, 1999). In both species we observed that at suboptimal temperatures, maximum heart rate (f_{Hmax}) of oil-exposed individuals was lower than in control specimens. Previously, Milinkovitch et al. (2013) have found in juvenile golden grey mullet exposed to crude oil (0.07 g L^{-1}, ∑PAH 3.3-60.1 µg L^{-1}) that the capacity of ventricular muscle strips to generate force at suboptimal temperature (14.5°C) and high stimulation frequency (1.2 Hz) was not affected when measured directly after exposure. Furthermore, these authors reported that oil exposure did not significantly affect the force-frequency relationship established in control, unexposed fish. Similarly, Nelson et al. (2016) found that a 24h exposure to PAHs (9.6 µg L^{-1}) did not influence the routine heart rate of adult mahi-mahi when measured directly after exposure. However, a significant decrease in routine cardiac output was reported by these authors, probably resulting from a decreased stroke volume. Claireaux and Davoodi (2010) found that juvenile common sole had reduced cardiac output, as well as impaired cardio-respiratory responses to an acute warming, 24h after exposure to crude oil (∑PAH 39 ng L^{-1} of water and 15–71 ng g^{-1} dry liver tissues). These authors found that 24h post-exposure there was no statistically significant difference in routine heart rates between oil-exposed and control sole at temperatures between 15-20°C (optimal temperature in sole is 19°C; Lefrançois and Claireaux 2003). However, a difference progressively appeared when temperature increased above 20°C with the cardiac output of the oil-exposed common soles gradually losing its capacity to match the temperature driven increase in metabolic oxygen demand (Claireaux and Davoodi 2010). There are no other

studies about the effect of crude oil or PAHs on heart rate in non-embryonic fish. However, work on embryonic fish indicates that exposure conditions affect the cardiac response to oil compounds. For example, developing zebrafish (*Danio rerio*) exposed to oiled gravel effluent displayed reduced routine heart rate, while exposure to water-accommodated fractions containing dispersed oil microdroplets had no effect (Jung et al. 2013). In Pacific herring (*Clupea pallasi*), oiled-gravel exposure increased the variability of routine heart rate and generally reduced it (Incardona et al. 2009). A similar response was observed in herring embryos exposed to bunker fuel after *Cosco Busan* collision in San Francisco Bay (Incardona et al. 2012), as well as in haddock (*Melanogrammus aeglefinus*) embryos exposed 0.7–7 μg L^{-1} ∑PAH (Sørhus et al. 2016) and in rainbow trout larvae exposed to 1.6 g L^{-1} of crude oil (Vosyliene et al. 2005). An exposure to ∑PAH ~2-5 μg L^{-1} (which is similar to the PAH concentration in our experiment with rainbow trout) reduced routine heart rate in embryos of bluefin and yellowfin tuna (Incardona et al. 2014). In larval red drum (*Sciaenops ocellatus*) the exposure to crude oil (2.6 μg L^{-1} ΣPAH) reduced the routine stroke volume but did not affect heart rate (Khursigara et al. 2016). In the current study, the reduction of f_{Hmax} at suboptimal temperatures in the oil-exposed fish was rather small (~4 bpm). Thus, it seems that the juvenile fish might be more resistant against oil-exposure than earlier life stages. In embryonic and larval fish significant reductions in heart rate and arrhythmias are seen already after nominal exposure to PAHs (e.g. Thomaz et al. 2009; Incardona et al. 2009, 2012, 2014; Jung et al. 2013; Sørhus et al. 2016; Khursigara et al. 2017). Furthermore, exposure in embryonic stages may have extremely long lasting effects e.g. on swimming capacity (Hicken et al. 2011; Mager et al. 2014) which could have consequences on the ecology of whole species.

In the current study despite the fact that f_{Hmax} was lower in exposed fish than in control fish at temperatures below the optimal temperature (T_{OPT}) of the species considered, this difference disappeared at temperature above T_{OPT}. As a result, the highest f_{Hmax} values were similar in exposed and control fish. Thermal tolerance of cardiac function i.e., T_{PEAK} (temperature where highest f_{Hmax} was observed) and T_{ARR} (temperature where arrhythmias were observed) were also higher in exposed rainbow trout than in control trout. In seabass, T_{PEAK} was also higher in exposed than in control seabass. Furthermore, in both species Arrhenius break point temperature (T_{AB}), which is indicative of the species' optimal temperature (e.g. Casselman et al. 2012; Anttila et al. 2013a; Ferreira et al. 2014), was higher in oil-exposed animals than in control ones. In general, these findings show that oil-exposure does not have significant negative impact either on cardiac function at high temperatures or the thermal tolerance of cardiac function. These are important findings, as in several species at high temperatures the maximum cardiac function is critical for fish survival through preserving aerobic capacity (Steinhausen et al. 2008; Farrell 2009; Eliason et al. 2013). A 4-month field experiment recently illustrated the ecological significance of these results as the survival and growth of juvenile oil-exposed seabass (0.07 g L^{-1}) was shown to be similar to that of control fish although they were exposed to naturally high summer temperatures (Mauduit et al. 2016). Taken together, our heart rate results indicate that oil exposure does not have significant lasting negative impact on the f_{Hmax} on juvenile fish when fish are tested after sufficient recovery time following exposure. Again, we cannot be sure if this is because of the recovery capacity or because used crude-oil concentrations do not have impact on maximum heart rate in the first place. Previously, Johansen and Esbaugh (2017) have shown in adult red drum (*Sciaenops ocellatus*) that acute oil exposure (12.1 μgL^{-1} ΣPAH_{50}) reduced the swimming capacity as well as aerobic scope and that these effects were still seen 6 weeks post exposure. We can therefore assume that oil-exposure in the current study did not have significant effect

on f_{Hmax} in the first place. However, it cannot be ruled out that other aspects of cardiac function, like stroke volume, would have been influenced as seen in mahi-mahi (Nelson et al. 2016).

Our values for heart rate and upper thermal tolerance lie within the range of published values from these animals. Rainbow trout swimming at maximum sustainable velocity at 15°C has a heart rate of 96 bpm after vagotomy (Priede 1974). This value compares nicely with the values of 80 bpm observed in the present study (see also Mercier et al. 2000). The maximum heart rate of exercising seabass has been measured at around 90 bpm at 20°C (Chatelier et al. 2005) which agrees relatively well with the current finding of 90 bpm at 17°C. The T_{AB} values (15.1±0.4°C and 19.3±0.3°C for rainbow trout and seabass, respectively) are somewhat lower, but near the optimum growth temperatures of these species (16.5-17°C and ~22-24°C, Jobling 1981; Claireaux and Lagardère 1999).

The two experiments that are reported here were conducted separately and differences in the experimental conditions and design must be considered, i.e., PAH composition of the oil tested, duration of the post-exposure recovery, concentration of crude oil tested, usage of dispersant and availability of tissue PAH concentrations.

Crude oil used in the seabass and trout experiments was of different origin and this was particularly reflected in their composition of PAHs, the most potent oil compounds from the cardiac standpoint. In embryonic fish it has been shown that PAHs are particularly detrimental to the cardiac function although reported effects are compound-specific (see review by Incardona and Scholz 2016). For instance naphthalene, chrysene, anthracene and benzo[k]fluoranthene, abundant PAHs in both tested oils, are known for having only small

effects on the physiology (conduction properties) and anatomy/pathology (pericardial edema) of the heart (Incardona et al. 2004; 2011). On the other hand, dibenzothiophene and phenanthrene have been shown to cause pericardial edema and to reduce heart rate by blocking atrioventricular conduction in a concentration-dependent manner (Incardona et al. 2004). Furthermore, benzo[e]pyrene and benzo[a]pyrene have also been shown to induce pericardial edema (Incardona et al. 2011). In the current experiments phenantherene was present in the water and fish tissues and dibenzothiophene was even the most abundant PAH in the seabass experiment. Thus, from this stand point, both oils contained PAH compounds that should have had influence on cardiac function.

Another difference between the two experiments is the duration of the post-exposure recovery period. The rainbow trout measurements were done one week after the exposure while seabass were measured 6 months post-exposure. The short-term effects of oil exposure upon the cardiac function are well documented (e.g. Incardona et al. 2009, 2012, 2014; Claireaux and Davoodi 2010; Jung et al. 2013; Milinkovitch et al. 2013; Brette et al. 2014; Nelson et al. 2016; Sørhus et al. 2016; Khursigara et al. 2017). A review of the literature shows, however, that published studies of the toxicological impacts of oil exposure on fish predominantly investigated acute exposure, short exposure duration and immediate assessment, mostly at low biological organization levels. While these studies contribute to increase our mechanistic understanding of contamination and decontamination processes (e.g. Ramachandran et al. 2004; Milinkovitch et al. 2013; Danion et al. 2014; Dussauze et al. 2014; Sadauskas-Henrique et al. 2016; Sandrini-Neto et al. 2016; Sanni et al. 2016), they are of limited help when addressing issues such as, for instance, impact on the resilience, health, production and recruitment of the affected populations (Forbes et al. 2006). From this perspective, the present experiments are remarkable as, although providing little additional

information about the well documented patterns of PAH bioaccumulation and metabolization in fish (Claireaux and Davoodi 2010; Milinkovitch et al. 2013; Nelson et al. 2016), they provide evidence in favor of the absence of chronic latent effects of oil exposure on fish thermal tolerance and cardiac performance. These results substantiate the recovery process reported by Claireaux et al. (2013) and Mauduit et al. (2016) regarding swimming capacity, tolerance to heat and to hypoxia. Interestingly, although the two sets of experiments involved two different fish species with quite different exposures and at two different times post-exposure, very similar response patterns were observed. This is an important result, because it shows that cardiac recovery could be a relatively general phenomenon in juvenile fish. However, in order to evaluate the recovery process more thoroughly, more time points should be examined in future.

One other difference between the two studies was that we didn't use dispersant in the trout study and that tissue PAH concentrations were not measured in that species. Nevertheless, nominal concentrations tested in present studies are representative of the concentrations measured following an accidental spill (Boehm and Fiest 1982; Milinkovitch et al. 2011), which have been shown to result in detrimental effects on cardiac function when measured within days-weeks post-exposure (e.g. Claireaux and Davoodi 2010; Incardona et al. 2014; Sørhus et al. 2016; Khursigara et al. 2017).

In conclusion, exposure to crude oil did not have a significant impact on maximum heart rate and cardiac function of juvenile fish at temperatures above their optimum when the measurements were done 1 week (trout) to 6 months (seabass) after the exposure. We observed that exposure may even improve the upper thermal tolerance of fish. The reason for this unexpected result is unknown, thus, future studies should focus on this issue. Our

findings also suggest that recovery processes exist that can reverse the functional impairments observed shortly following direct exposure in previous studies. This is also an important finding for the fisheries as both of these species are important human food species and are living/reared at areas where the threat for both oil accidents and warming surface waters is particularly high.

Figure legends

Fig. 1 The cardiac responses to increasing temperature in control and oil-exposed fish after one-week (rainbow trout) and six-month (seabass) recovery. The effect of increasing temperature on the maximum heart rate (f_{Hmax}) of **a** juvenile rainbow trout and **b** European seabass. The grey area indicates the temperature window where there are significant differences between control and oil-exposed fish. The dotted lines indicate temperatures where some of the fish already had arrhythmias. The Arrhenius plots of f_{Hmax} responses to increasing temperature for rainbow trout are given in **c** and for seabass in **d**. The vertical lines (dotted line for oil-exposed fish, solid line for control fish) indicate an Arrhenius break point temperature (T_{AB}). *n* = 12 for rainbow trout and *n* = 18 for seabass

Fig. 2 The temperature tolerance values of control and oil-exposed fish. Arrhenius break point temperature (T_{AB}), temperature for peak maximum heart rate (T_{PEAK}), temperature where cardiac arrhythmias are observed (T_{ARR}) and critical upper temperature tolerance (CT_{MAX}) of **a** rainbow trout and **b** European seabass. * indicates statistically significant differences between oil-exposed and control fish. *n* = 12 for rainbow trout (except for CT_{MAX} measurements *n* = 15) and *n* = 18 for seabass (except for CT_{MAX} measurements *n* = 20)

Compliance with ethical standards

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure 1

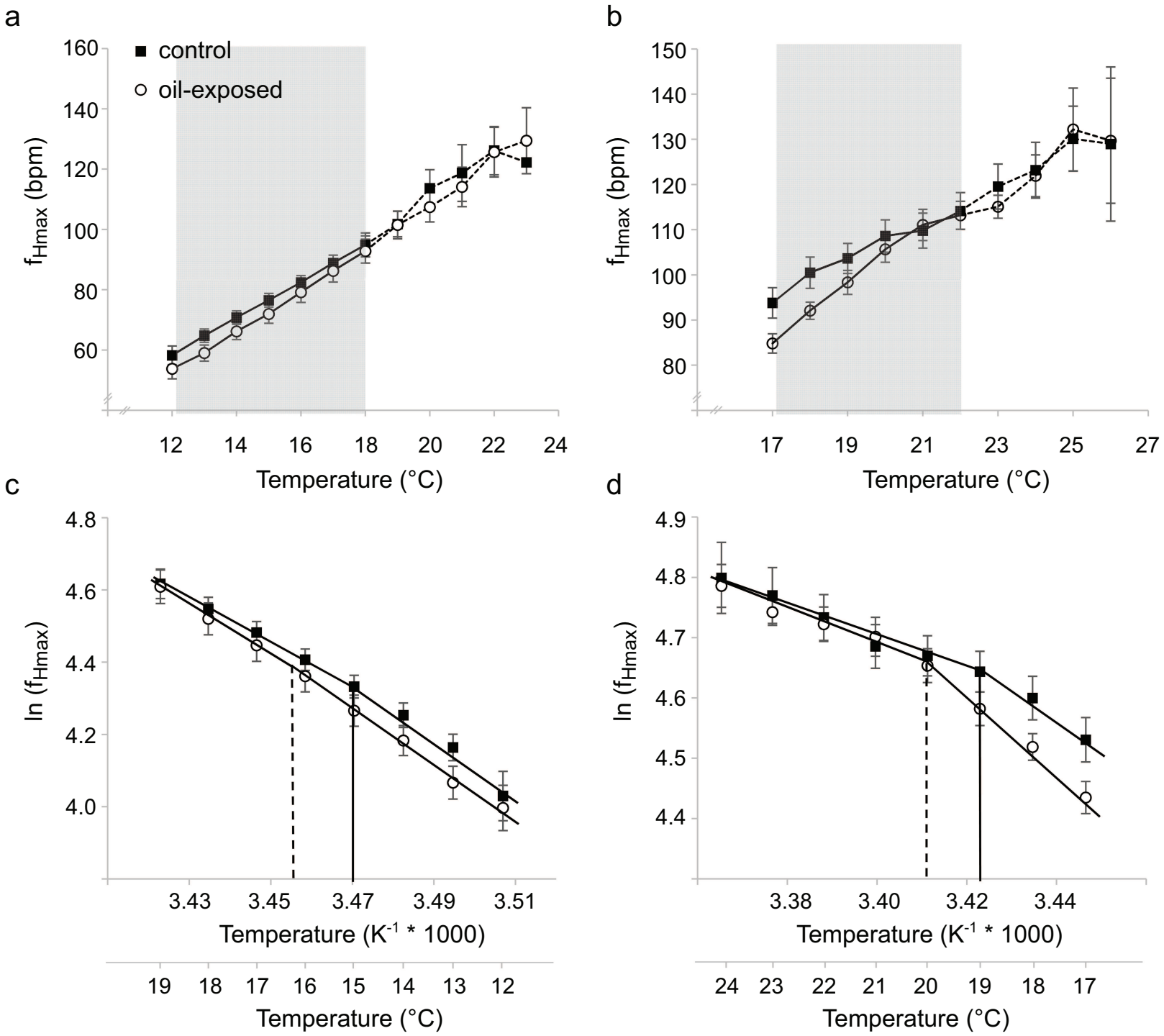
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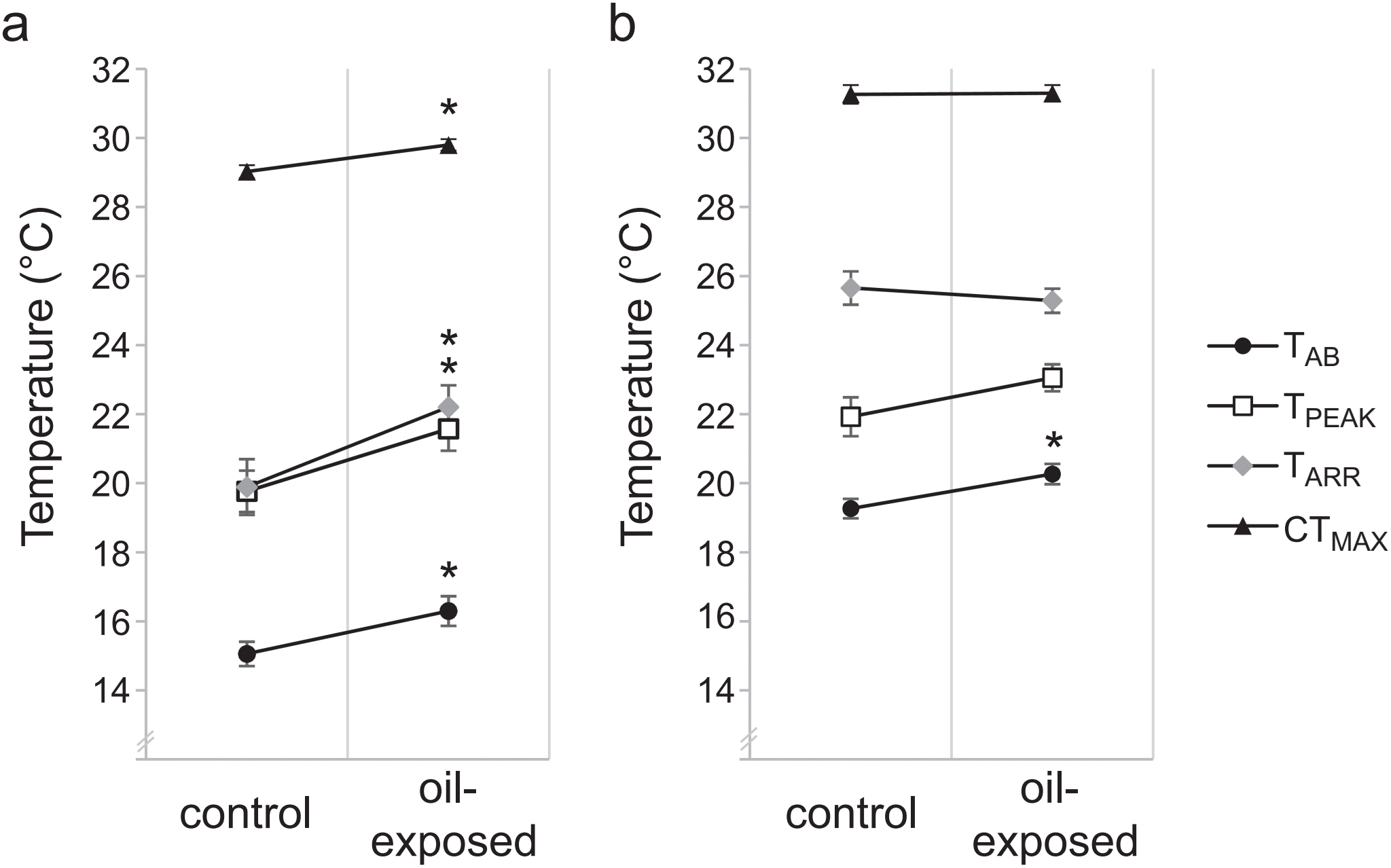
Figure 2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