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ORIGINAL RESEARCH PAPER

Evolution of pace-of-life syndrome under conditions of maternal PCB contamination and global warming in early life stages of cold stenothermic fish (Arctic char)

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ABSTRACT

The end of the 20th century was characterised by rapid modifications of ecosystem functioning under different pressures (such as eutrophication and toxic pollution). Increasing temperatures in the context of global warming could have indirect consequences, such as increased bioavailability of hydrophobic organic pollutants among aquatic species. According to the “pace-of-life syndrome” (POLS) theory, these stressors could lead to covariations in many life traits. Lake Bourget is the largest natural lake in France and has been highly polluted from the fifties to the eighties both with a high load of nutrients (wastewater discharge) and polychlorinated biphenyls (PCBs) (industrial effluent discharge). Despite improvements in water quality since the 21st century, PCB levels are still higher than the United States Environmental Protection Agency cut-off for wildlife protection. The population of Arctic char, a cold stenothermic salmonid, has remained low in Lake Bourget for the last ten years despite restocking efforts and complete re-oligotrophication. We hypothesised that PCB pollution can affect the Arctic char population and that the increase in water temperature could magnify the effects of PCB. Thus, this study aimed to investigate the effects of maternal PCB contamination on offspring using a multiparametric and multiscale approach. Female Arctic char were contaminated with PCB before spawning, and each fertilised spawn was incubated at two temperatures (4 and 8.5°C). The results showed that co-exposure to increased temperature and maternal PCB contamination influenced biodemographic, physiological, and behavioural parameters. The effects were highly dependent on the developmental stage. Based on the POLS theory, a continuum of life traits that may reflect potential physiological and behavioural modifications in response to these concurrent stressors is highlighted

Keywords: PCB, temperature increase, concurrent stress, POLS, life history

1 INTRODUCTION

Anthropogenic pressures are increasingly affecting aquatic organisms via physicochemical stressors, including decreased oxygen levels, xenobiotics, and poor water quality. The question arises as to the adaptive capacity of species to cope with these multiple modifications in a short period. Organisms will need to alter their phenotype in response to these changes. The pace-of-life syndrome (POLS) theory (Réale et al., 2010) suggests the presence of covariations in many life-history traits collectively referred to as POLS. Réale et al. (2010) suggested that there are covariations in life-history traits characterised by biodemographic strategy, behaviour, and physiology in a defined environment according to a fast/slow pace of life continuum. Indeed, when the environment changes, all life-history traits change along the same trajectory (Goulet et al., 2017). For instance, the acceleration of metabolism is linked to an increase in temperature, which leads to an increase in detoxification mechanisms; this could affect the set of life traits (Réale et al., 2010) and shape sensitivity to contaminants (Goulet et al., 2017).

Typically, two behavioural profiles of individuals are observed according to their responses to stressful situations: (1) reactives have a freeze/hide response with immobility behaviours and low levels of aggression (Engel and Schmale, 1972) and (2) proactives have a fight/flight response and display territorial and aggressive behaviours and low monoaminergic system reactivity (Øverli et al., 2010) and lower neuronal plasticity compared to reactive individuals (Øverli and Sorensen, 2016). Nevertheless, it is important to note that in natural and experimental populations, most individual stress responses lie between these two extremes.

The impact of the environment on the evolution of syndromes (covariant life-trait associations) may extend beyond its current state to subsequent ontogenetic stages or generations (Sih et al., 2004). Indeed, the long-term effects of stress exposure in early prenatal and postnatal life can lead to an increase in glucocorticoid hormone levels and influence stress responses in adulthood (Monaghan and Haussmann, 2015). Hormones involved in stress pathways play an important role in homeostatic energy management and have been proposed to underlie the mechanisms by which offspring are made aware of the postnatal environment (Sheriff and Love, 2013).

Individuals exposed to stress early in life may exhibit phenotypic changes in their behaviour (Monaghan and Haussmann, 2015). Prenatal exposure to stress hormones can also produce negative effects by inhibiting the escape response to predators in the F1 generation, as seen in three-spined sticklebacks (*Gasterosteus aculeatus*) (McGhee et al., 2012). Among stressors, the effects of temperature have been particularly emphasised in early life stages, partly due to their immature homeostatic capacity (Urho, 2002).

Moreover, contaminants may directly or indirectly interfere with physiological systems, for example, through the inhibition of acetylcholinesterase (AChE) (Sarkar et al., 2006) or the generation of oxidative stress (Lushchak, 2011). Moreover, oxidative stress is known to mediate life history trade-offs (Janssens and Stoks, 2018), sensitivity to oxidative stress is integral to POLS, and high sensitivity is associated with a rapid rate of life history. Also, an increase in temperature can produce indirect effects, such as increased bioavailability of hydrophobic contaminants by desorption of particulate solids (Ma et al., 2011). In addition, owing to their lipophilic characteristics, PCB accumulate in eggs via the yolk sac, allowing vertical transmission of the xenobiotic from one generation to the next, and thereby causing very early exposure in the next generation. This exposure may also lead to a reduction in larval survival and physiological and behavioural changes (Péan et al., 2013). Although the effects of thermal stress have been widely reported, interactions between thermal and chemical stresses have only more recently been studied (Gandar, 2015). Different effects of this combination have been described, including antagonistic, additive, synergistic, and potentialising effects. Exposure to pollutants will likely lead to a decrease in the thermal tolerance of fish, accentuating the effects of combined stress.

Thus, concurrent stress could potentially alter the relationship between behaviour and life history within POLS. According to the POLS hypothesis, animals on the fast end of the continuum invest less in functions that are not directly related to growth and require considerable energy for detoxification and repair mechanisms (Congdon et al., 2001).

Among the species at risk, the Arctic char (*Salvelinus alpinus*), a cold-water salmonid, has heritage, cultural, and economic importance throughout North America and Europe, particularly in large peri-alpine lakes (Caudron et al., 2014). In these ecosystems, the Arctic char is at the southern limit of its range and considered a sentinel species of pre-alpine lake quality. In peri-alpine lakes, the Arctic char showed a sharp increase in its population at the beginning of the 20th century before showing a decrease starting in the 1990s, particularly within Lake Bourget, despite the existing policy of supportive breeding stocking practices and lake re-oligotrophication (Caudron et al., 2014). Tierney et al. (2010) suggested that the indirect effects of environmental pollutants were responsible for this decrease. The concentration of the 7 indicators of PCB pollution in lake Bourget water was found to be as high as 0.46 ng/L by Naffrechoux et al. (2017). Despite the ban on PCB use since 1987 in France, Arctic char contamination still reached values of 200 ng for 6 PCB/g ww, thus making it unsuitable for human consumption (European standard < 125 ng/g).

We hypothesised that PCB pollution can affect the Arctic char population and that an increase in water temperature could magnify the effects of PCBs. Using a multiparametric approach, the aims of this study were to (1) evaluate whether increased temperature and maternal PCB exposure act as potentialising, synergistic, additive, or antagonistic factors on life history traits and (2) determine whether life history traits vary concomitantly following increased temperature and maternal PCB contamination by considering the different rhythms of life proposed by the POLS theory.

2 MATERIALS AND METHODS

2.1 Experimental design

Arctic char (*Salvelinus alpinus*) (mean age, 5 years) were obtained from a brood stock on a fish farm (Charles Murgat, FR38032001CE): nine females (body weight, 3.7 ± 0.9 kg and total length, 42.40 ± 1.7 cm) and ten males (body weight, 1.1 ± 1.2 kg and total length, 40.1 ± 1.1 cm). The females were divided into groups of three individuals and received a single intraperitoneal injection of PCB (Aroclor 1244 dissolved in corn oil) one month before egg laying; the first group received an injection of corn oil (control group), the second group received an injection of 500 ng/g (moderate) and the third group received an injection of 1 000 ng/g (heavy) (table 1). The doses were similar to the concentrations measured in wild Arctic char from Lake Bourget (Naffrechoux et al., 2017). Oocytes were obtained one month after stripping. PCB levels were measured in female fish muscle after spawning and in the oocytes. The concentrations of seven PCB were analysed using a gas chromatography/pulse discharge ionisation detector after ultrasound extraction according to the protocol published by Masset et al. (2019). A calibration curve was constructed using an internal standard for the determination of PCB concentrations in the oocytes. Oocytes from each female were individually fertilised with a pool of milts from ten males. Eggs were incubated in six similar recirculating water systems or incubators, divided into two thermostatic chambers. In each incubator, temperature was checked daily (precision of the probe ODEON, ± 0.1°C) and recorded continuously (Minidot sensor, accuracy ± 0.1°C). Dissolved oxygen levels were also measured and remained above 90 ± 10%. The total ammonia and nitrite concentrations in each incubator were kept below 0.05 and 0.01 mg/L, respectively, and the pH remained at 8.0 ± 0.4. Each thermostatic chamber was randomly assigned to the different experimental temperatures: (1) 4°C and (2) 8.5°C based on a global warming scenario according to Representative Concentration Pathway 8.5 (Intergovernmental Panel on Climate Change [IPCC], 2014), as described in supplementary data 1. Thus, the effect of an increase in temperature alone was estimated in our control group incubated at 8.5°C. The effect of PCB contamination was estimated in individuals incubated at 4°C (reference temperature according to Mari et al., 2021). Finally, the effects of the combination of the two stressors (temperature and PCB) were investigated among individuals from contaminated mothers incubated at 8.5°C. After mixed feeding, the fry were provided with pellets *ad libitum*.

Five biological stages were investigated: (1) hatching, defined as the time during which at least 50% of the fry had left their egg shells, (2) emergence as the time when at least 50% of the fry rose to swim in the water column, (3) mixed feeding at the time when at least 50% of the fry started to feed exogenously and fry still had yolk sacs, (4) exogenous feeding at the time when at least 50% of the fry had experienced total yolk sac absorption, and (5) juvenile as the time when at least 50% of the fry had juvenile-like phenotypes (Réalis-Doyelle et al., 2017). Five developmental periods were assigned among these biological stages: (1) P1: from fertilisation to the hatching stage, (2) P2: from hatching to the emergence stage, (3) P3: from emergence to the mixed feeding stage, (4) P4: from mixed feeding to the exogenous feeding EF stage, and (5) P5: from exogenous feeding to the juvenile stage.

2.2 Biodemographic parameters

The global development time for each condition was given at the end of the experiment as the number of days between the fertilisation and juvenile stages, both in days and degree days.

Unfertilised eggs were not considered dead and were excluded from the analysis. Dead individuals (embryos or fry) were counted every day to calculate the daily mortality rate (DMR, in percentage) over a period of time, as follows: [DMR = ((Number of dead individuals cumulated over a period of time / Number of days within this period of time) × 100 / Total number of individuals]. Based on these data, the mortality rate was obtained and we then calculated the survival rate (SR). The survival rate (SR) was calculated for each condition (from fertilisation to juvenile) and at each stage:

$$SR = \frac{100\ -\ (\text{Number of dead individuals cumulated over a period of time}\ \times\ 100}{\text{Number of individuals at the beginning of the studied period of time}]}$$

Fish morphology was observed using a camera (Nikon D5300; Zoom Sigma 105 mm F2.8 EX DG Macro 06). Observations were made on 150 live fry from each female (n = 30 per stage) at the end of each period (5 times) for all subsequent traits. The fry were measured using IMAGE J software (National Institutes of Health, Bethesda, MD, USA) with a precision of almost 0.01 cm. Seven morphometric characteristics were chosen to calculate allometric growth patterns in Arctic char during development: total length (TL), measured as the distance from the snout to the tip of the tail; head length (HL), as the distance between the tip of the snout and the opercula edge; head height (HH), as the larger height of the head measured perpendicularly to the midsection of the eye; eye diameter (ED), as the mean of the maximum and minimum diameters of the eye orbit; jaw length (JL), as the distance between the lower jaw and the opercula edge; trunk length (TaiL), as the distance between the edge of the operculum and the anal opening; myotome height (MH), as the distance perpendicular to the body axis between the anus and the base of the dorsal fin; and tail length and caudal fin height (CaH), as the distance perpendicular to the axis of the body between the upper and lower ends of the caudal fin (figure 1).

Fry with visible deformities, including malformations of the vertebral column or yolk sac were categorised as malformed larvae. The malformation rate was calculated as follows:

$$\mathrm{MR}= \frac{\text{number of malformed larvae} \times 100}{30}$$

All the larvae were photographed and used for morphometric measurements.

Specific growth rate (SGR) was used as a proxy for metabolic rate performance parameters, and calculated as follows:

$$\mathrm{SGR} = \frac{\mathrm{Ln(TL\ to\ D_f) - Ln(TL\ to\ D_i) \times 100}}{\mathrm{D_f - D_i}}$$

(Trabelsi et al., 2015), where D_i and D_f represent the initial and final days, and Ln is the natural logarithm.

2.3 Behavioural parameters

Behavioural analyses were performed at the juvenile stage for all treatments. Prior to the tests, an acclimation period of 5 min was applied. For each test, six individuals were filmed for 15 min. The set of tests was performed in triplicate for each condition with new individuals. All behavioural tests were performed in a circular arena (diameter, 30 cm; height, 10 cm; and water depth, 6 cm). The water used for the behavioural analysis was the same as that used for each conditioning session. The bottom of the arena was transparent and lit at 5–10 lx on the side to avoid shading of the larvae during recording. Aggression was defined as the number of agonistic interactions that an individual had with others. The number of the contacts was calculated as follows: (type of contact × 100)/total number of contacts.

Activity time was recorded in two categories: (1) static swimming (a tail stroke, but which did not propel the fry beyond their body length for > 5 s) and (2) fast swimming (more than one body length in < 5 s). Three types of activity were defined: (1) jerky swimming < 5 s, (2) fast swimming > 5 s, and (3) inactivation when the larvae showed no swimming activity. The percentage activity was calculated as follows: (time of activity type × 100)/total time.

Anxiety is a trait that is measured in teleosts for both larvae and later stages (Levin et al., 2007) by placing fish in a new environment. Larval positions in the arena were scored based on four categories: (1) high position in the arena centre, (2) low position in the arena centre, (3) periphery of the high arena, and (4) periphery of the low arena. The percentage of time spent per zone was calculated as follows: (time spent in the position in the arena × 100)/(total time in the arena).

2.4 Morphological parameters

Two types of measurements were obtained: (1) between each biological stage and (2) between the hatching and juvenile stages. Using IMAGE J software, the body perimeters (PC, mm) and yolk sac (PSV, mm) were measured, and body areas (AC, mm²) and yolk sac areas (ASV, mm²) were computed. In addition, the efficiency of yolk use was calculated (EUV) as proposed by Hardy et al. (2004):

$$\mathrm{EUV} = \frac{\text{AC at } D_f - \text{AC at } D_i}{\text{ASV at } D_i - \text{ASV at } D_f},$$

where AC represents the body area, ASV represents the yolk sac area, and D_i and D_f represent the initial and final days, respectively. All measurements (n = 30 fry per female/temperature) were obtained from well-formed fry with an accuracy of 0.01 cm.

2.5 Stress enzymes

For each of the three analyses, five larvae were ground in a 100 mM sodium phosphate buffer at pH 7. After a short centrifugation step, enzyme activity was determined in the supernatant. Stress biomarker assays of different markers of oxidative stress (superoxide dismutase [SOD]) and neurological stress (acetylcholinesterase [AChE]) were performed according to Noury et al. (2016a, b). Catalase (CAT) levels were measured using a spectrophotometric method according to Li and Schellhorn, (2007).

2.6 Statistical analysis

The distributions of data were determined using the Kolmogorov–Smirnov test, and the homogeneity of variances was tested using Levene’s *F* test. In all statistical analyses, temperature, oocyte PCB contamination, and biological stage were test factors. PCB levels in oocytes were analysed using t tests.

A global analysis was performed to evaluate the effects of temperature and its interaction with PCB contamination on SR, development time, malformation rate, oxidative (SOD, CAT) and neural (AChE) stresses, metabolic performance parameters (yolk sac efficiency and SGR), in addition to behavioural parameters. For this measurement, a generalised linear model (GLM) was used ($glm2$ R package) ; temperature and PCB contamination were specified as fixed effects, while female identity was defined as a random effect. A second analysis was then performed, considering the stage for the same parameters used for the GLM; temperature and PCB contamination and biological stage were specified as fixed effects, while female identity was defined as the random effect. The analysis was followed by a post-hoc Tukey test to calculate pairwise differences between means when the interaction between factors was significant. All parameters are expressed as the mean ± standard error.

For each morphological characteristic, the ratio (data of body characteristic/total body length) was used. At each temperature, multiphasic growth was described via regression estimated from logarithm-transformed data for each morphometric parameter. To perform this procedure, the relationship to TL was calculated with the following allometric growth model put forward by Fuiman (1983): $log(Y) = log(a) + b\ log(TL)$, where Y represents the given characteristic under study for which a is the intercept, and b is the slope (allometric growth coefficient). These inflexion points on the growth curves designated the value of the body character where the regression slopes changed and were determined according to the method described by Van Snik et al. (1997), in which the $(x - y)$ data set was sorted according to increasing TL. The inflexion point corresponded to the coupled $x - y$ that resulted in the largest t value when comparing the growth coefficients (b) using Student t tests.

A Student t test principal component analysis (PCA) was performed using the resultant eigenvectors of the PCA for the measured characters (Ben Khemis et al., 2013; Réalis-Doyelle et al., 2017). It is generally accepted that when groups of individuals with different growth patterns are included in the PCA, PC1 summarises the shape variation resulting from growth allometry, while PC2 summarises the variation in divergent growth trajectories. Hence, growth patterns among different growth phases were reflected as divergent PC2 trajectories when plotted against PC1. Piecewise linear regression, fitted with a nonlinear procedure, was used to estimate changes in PC2 orientation: $PC2 = b_0 + b_1 TL + b_2(TL - Lm)\ (TL \geq Lm)$, where b_0 is the intercept, b_1 is the slope determined during the “fry” stage, b_2 represents the difference in slope between the “fry” (corresponding to first growth phase) and “juvenile” stages (corresponding to second growth phase), and Lm represents the length of morphometric metamorphosis, which corresponds to TL at which slope changes and growth coefficients equals (i.e. isometry) (Nikolioudakis et al., 2010; Ben Khemis et al., 2013). The PC1 eigenvector from groups of individuals sharing common growth patterns reflected the relative proportion of changes. Thus, the variable was isometric when its PC1 component score equalled $\frac{1}{\sqrt{p}}$, where p is the number of variables (LH, HH, ED, HM, JL, TaiL, and CaH) in the analysis. A bootstrap method was used to estimate the standard errors and confidence intervals for component score comparisons with the theoretical isometric value (Ben Khemis et al., 2013). The *vegan* 2.0–9 R package was used for the PCA, *boot* 1.3–9 was used for bootstrapping, *Nortest* 1.0–2 was used to analyse the normality of distributions, and *SiZer* 0.1–4 was used to estimate inflexion points via partial likelihood ratio tests.

All statistical analyses were performed using R version 3.6.2 (R Development Core Team, Vienna, Austria). All results were considered statistically significant at $p < 0.05$.

3 RESULTS

PCB concentrations in the oocytes and muscles were measured for each female in each group. The *t* test results showed a significant difference among the three groups. The "control" group, corresponding to females injected with oil only, showed PCB contamination corresponding to 50 ± 5 ng/g wet weight in oocytes and 7.6 ± 0.2 ng/g wet weight in muscles. The "moderate" group, corresponding to females injected with 500 ng/g, showed PCB contamination of 102 ± 11 ng/g wet weight in oocytes and 48.6 ± 7.4 ng/g wet weight PCB in muscles. The "heavy" group, corresponding to females injected with 1 000 ng/g, showed contamination of 239 ± 10 ng/g wet weight in oocytes and 78.9 ± 6.1 ng/g wet weight in muscles (table 1). The mean muscular concentration found in individuals from Lake du Bourget was 193 ng/g wet weight of PCB (supplementary data 2). For all results, the "female" effect was not significant ($p < 0.05$).

3.1 Biodemographic parameters

The total time from fertilisation to the juvenile stage was significantly different between temperatures (GLM: $F = 9.51$, $p < 0.01$) and decreased with increasing temperature (4°C for the control group: 170 ± 2 days; for the moderate condition: 170 ± 1 days and heavy condition: 169 ± 2 days; at 8.5°C for the control group: 112 ± 2 days, in the moderate condition: 108 ± 2 days, and heavy condition: 100 ± 2 days) (figure 2).

SRs differed significantly according to the interaction between PCB contamination and the developmental period (table 2 and supplementary data 2). The most critical period for larvae in the “heavy” group was the P1 period, as shown in supplementary data 2, for which peak mortality was observed between 15 and 29 days post-fertilisation (blastula stage), depending on the test temperature and PCB contamination (table 2). This effect was more significant at 4°C in the heavy group; however, a decline in SR was also observed at 8.5°C, with an SR of 12.5% for juveniles. This high contamination had also more impact at 4°C, as seen from an SR of 23.5% among juveniles (supplementary data 2). For each temperature, we found two phases corresponding to the two growth phases based on the length of morphometric metamorphosis (Lm), summarising the profiles of morphological changes during development. For each condition, an Lm was found (at 4°C: for the control group, Lm = 1.65 ± 0.45 mm; for the moderate group, Lm = 1.67 ± 0.42 mm; and for the heavy group, Lm = 1.44 ± 0.12 mm; at 8.5°C: for the control group, Lm = 1.71 ± 0.27 mm; for the moderate group we did not observe any growth shift; and for the heavy group, Lm = 1.47 ± 0.34 mm (supplementary data 3). Examination of the residuals from the fitted bivariate allometric equations revealed a structured (non-random) distribution for all morphometric characteristics examined (ED, HH, HL, JL, MH, TaiL, and CaH) in relation to TL, indicating a shift in their relative growth (supplementary data 3).

Estimates of the PC1 component coefficient were quite stable within both phases (1 and 2), as indicated by their small ranges, thus demonstrating the robustness of the groups generated by the Lm values provided by the PCA (supplementary data 4). Body proportions changed considerably during the pre-larval and larval stages. The change in growth was defined via mathematical calculation. In the piecewise linear regression, different phases of growth indicated a positive allometric relationship with TL for the head variables (HL, HH, and ED), MH, and trunk length during phase 1 of growth.

At 4°C, for the heavy group, the PC1 component coefficient for most of the parameters demonstrated isometric growth during the first growth phase; only the parameter CaH showed positive growth. The second growth phase showed the same pattern, except for MH, which showed negative growth (supplementary data 4). In contrast, at 8.5°C in the heavy group, most parameters related to head morphology showed isometric growth during the first growth phase, while MH showed positive growth and TaiL showed negative growth.

In addition, during the second growth phase, the PC1 component coefficient for MH showed negative growth. At 4°C, in the moderate group, the larvae exhibited positive growth for most parameters related to head morphology during the first growth phase. During the second growth phase, MH showed greater positive growth, whereas TaiL and CaH showed negative growth rates. In contrast, during the first growth phase, larvae incubated at 8.5°C in the moderate group showed characteristics related to horizontal growth and more specifically general somatic growth in terms of MH, TaiL, and CaH, all of which showed positive growth. In addition, during the second growth phase, most parameters showed positive and isometric growth in terms of head parameters. In contrast, at 4°C in the control group, all parameters showed positive growth during the first growth phase, and most of them showed isometric growth during the second phase. The same pattern was observed for larvae at 8.5°C during the first growth phase. During the second growth phase, most head and tail parameters and MH showed positive growth.

The malformation rate differed significantly according to the interactions among temperature, life stage, and PCB contamination level (table 2). The percentage of malformed fry increased in the moderate and heavy groups at all temperatures and stages (supplementary data 2). Under these conditions, most malformations (oedema) were observed (92%) in the yolk sac, and the other malformations observed occurred in the vertebral column (lordosis and scoliosis) (supplementary data 5). The calculated SGR was significantly affected by the interactions among temperature, life stage, and PCB contamination level (table 2). Also, at 8.5°C in the heavy group, larvae showed the highest SGR, and at 4°C, the SGR was highest in the heavy group (supplementary data 2).

3.2 Behavioural parameters

The number of physical interactions was significantly affected by temperature (figure 3), with a greater number of interactions at 8.5°C. The dichotomy of behaviour types showed a significantly different distribution between the two temperatures (χ^2 test $p < 0.05$). The escape of individuals in both groups occurred more frequently at 4°C, whereas at 8.5°C, during an interaction, only one of the two individuals escaped (figure 3). Overall activity, without consideration of swimming speed, was affected by PCB contamination (figure 3). Activity was significantly higher in the heavy group, with an activity time twice as high as that in the control group and greater saccadic swimming activity. The anxiety test showed that the position of the larvae in the arena was significantly affected by temperature and PCB contamination (table 2). Behaviours in the water column showed that the larvae took up different positions for the moderate and heavy groups at 8.5°C compared to other individuals that were preferentially at the bottom and periphery of the water column.

3.3 Physiological parameters

Yolk sac efficiency differed significantly according to the interactions among temperature, developmental stage, and PCB contamination (table 2). The yolk sac efficiency between hatching and MF decreased significantly under conditions of PCB contamination at 4°C for all stages of development. At 8.5°C, the lowest yolk sac efficiency was found in all stages in the moderate group (supplementary data 2).

Oxidative stress (as measured by CAT and SOD) differed significantly according to the interaction between temperature and PCB contamination (table 2). CAT activity was the highest during the entire development of the heavy group at 4°C (supplementary data 2). At 4°C, the oxidative stress marker, SOD, showed an increase during emergence in both the moderate and heavy groups. At 8.5°C, the highest SOD activity was observed at the EF stage in the moderate and heavy groups.

AChE activity differed significantly according to the interactions among temperature, biological stage, and PCB contamination (table 2). At 4°C, a strong decrease in AChE activity during hatching in the heavy group was noted, followed by an increase in AChE activity during the MF stage. At 8.5°C, AChE was strongly expressed in the heavy group during the hatching stage, after which it decreased during the MF stage and then increased again.

4 DISCUSSION

Using a multi-parametric approach, this study highlights the associated effect of PCB contamination and increased temperature during the early life stages of Arctic char. Furthermore, our study showed the synergistic effects of the two stressors at specific biological stages. This study partially supports the POLS theory by explaining the transition from the fast-slow continuum to the covariation syndrome.

4.1 PCB contamination

We found different levels of contamination between oocytes and muscles. These differences can be explained by the lipophilic nature of PCBs. Since oocytes are mainly composed of fat, PCBs preferentially accumulate in them. This phenomenon of differentiated accumulation of PCBs according to organ is referenced in the literature. In addition, this phenomenon of differentiated accumulation between oocytes and muscles has already been identified as potentially related to detoxification in females (Rypel et al., 2007).

4.2 Sole impact of temperature

Temperature alone affects the developmental time between stages, consistent with reports in the literature that developmental time is nonlinear (Kamler, 2002). Temperature affects the number of physical contacts between the larvae. This response has also been described by others authors (Colchen et al., 2016; Debecker and stoks, 2019).

4.3 Sole impact of PCB

The effects of PCB on activity can be explained by the combination of a high energy requirement for the maintenance of a fast life and efficient detoxification and repair mechanisms (Congdon et al., 2001).

4.4 Synergistic impact

In our study, PCB contamination and temperature showed a synergistic impact on a majority of biodemographic parameters.

Temperature and PCB contamination showed a synergistic effect on mortality in the heavy group at 8.5°C, a decrease in survival with only 12% of larvae surviving. Our study highlights this synergistic impact, particularly among larvae incubated at 8.5°C in the heavy and moderate groups, which showed the highest growth rates. The influence of temperature on growth is well known (Kamler, 2002; Réalis-Doyelle et al., 2016). Nikolioudakis et al. (2010) concluded that the synchrony (a brief change in growth) of allometric changes in morphological and osteological characteristics might be higher at low temperatures than at high temperatures. Moreover, our results showed a strong increase in malformations, mainly localised to the yolk sac and spinal cord. These malformations result from an increase in temperature among salmonids (Réalis-Doyelle et al., 2016) and PCB (Billsson et al., 1998).

Moreover, the synergistic effect of PCB contamination and temperature was observed in larvae and led to several physiological dysfunctions that may also explain some of our results. For example, our study showed an increase in oxidative stress markers (CAT and SOD levels) among juveniles in the moderate and heavy groups at 8.5°C due to the potentiating effect of PCB contamination on temperature. This increase in oxidative stress enzyme levels tends to confirm the shift from a slow to a fast pace of life, as suggested by the POLS theory (Réale et al., 2010). This increase in stress via an increase in temperature agreed with the literature describing salmonids (Simčič et al., 2015) and other species (Zhang et al., 2009). An increase in these oxidative stress markers following PCB contamination has been reported in goldfish

(*Carassius auratus*); nonetheless, Palace et al. (1996) did not observe a change in SOD and CAT activity in juvenile lake trout (*Salvelinus namaycush*) following exposure to different PCB concentrations via intraperitoneal injection. Thus, the developmental stage during which the experiments were conducted appears to be preponderant. Our study emphasises the synergistic effect of PCB contamination and temperature on yolk sac parameters, which are related to embryo-larval metabolism. Thus, when the temperature increases, metabolism and physiological requirements (respiration and maintenance) increase, which induces quicker consumption of the yolk sac (Palace et al., 1996) thus, less energy is proportionally available to form new tissues. This process could lead to the induction of heat stress defence systems, such as heat shock proteins or antioxidant enzymes, resulting in additional metabolic costs (Gandar, 2015).

Indeed, temperature and contamination have a synergistic effect on the neurotransmitter AChE, depending on the developmental stage. AChE activity increased with increasing temperature and contamination in the heavy group at 8.5°C. However, Laetz et al. (2013) did not observe an effect of increasing water temperature on AChE activity in juvenile Coho salmon (*Oncorhynchus kisutch*). Barra et al. (2001) observed the inhibition of AChE activity in chub (*Leuciscus cephalus*) exposed to a river heavily contaminated with PCB. Nevertheless, as shown by our study and the variability of results found in the literature, developmental stage also seems to have a prominent influence on the activity of this neurotransmitter.

Co-exposure to PCB contamination and temperature changes produced a potentiating effect on anxiety in the moderate and heavy groups at 8.5°C. Indeed, these larvae were the only ones present at the top of the water column, either at the centre or periphery. This positioning was in contrast to the anti-predatory behaviour described in teleosts (Flatt, 2005). This typical behaviour has been generally described in experiments in which individuals were subjected to anxiolytic molecules (Levin et al., 2007). Thus, because of their low anxiety levels, these larvae could be more easily exposed to predators. Conversely, individuals characterised as shy and/or more anxious are less exposed to predators. Furthermore, many studies have shown that behavioural syndromes are heritable (Debecker and Stoks, 2019) or believed to be modulated by underlying neuroendocrine and developmental mechanisms favouring particular behavioural combinations (Sih et al., 2012). This combination of traits can be linked to the theory that constraints on the allocation of time, energy, and/or tissue give rise to life-history trade-offs, and that endocrine control mechanisms produce incompatible physiological states, generating a restricted set of life-history outcomes (Ricklefs and Wikelski, 2002). Nevertheless, these observations could change over time because the personality of teleosts is not fixed in the larval and juvenile stages but appears to stabilise thereafter during the adult stage (Polverino et al., 2016) in the absence of external stressors.

4.5 Ecological impacts of the POLS theory

If our parameters are considered, although incomplete compared to the parameters used by Réale et al (2010), a continuum of three profiles can be distinguished (table 3). The control group showed a slow pace of life with minimal activation of stress enzymes, low anxiety, and active behaviour. The allometric development follows that described among salmonids (Réalis-Doyelle et al., 2017). Positive or near-isometric head growth for all conditions allows fry to catch prey, improve their respiratory capacity (Osse and Van Den Boogaart, 1995) and acquisition of vision (Packard and Wainwright, 1974). During the second growth phase, Arctic char must acquire hunting behaviour and improve locomotor performance and oxygenation to survive (Ben Khemis et al., 2013). Positive caudal fin growth and myotome height at 8.5°C in the control group were shown to improve propulsive speed and swimming efficiency by reducing energy costs (Ben Khemis et al., 2013). Propulsion development is crucial for both catching and avoiding predators (Osse and Van Den Boogaart, 1995). This maturation of the digestive system appeared to be confirmed by the positive head and tail growths that reflect the development of food capture and digestion structures in addition to changes in the respiratory system (Engel and Schmale, 1972). In conclusion, individuals in our study that were not contaminated with PCB approximated the profile by showing a slow life rhythm, as proposed by the POLS theory.

The moderate group showed the same growth profile as the control group during the first phase of allometric growth. Nonetheless, this group displayed a low activity rate, which could be due to poor conditions for minimising energy expenditure. Hypotheses pertaining to metabolic preservation when environmental conditions are unfavourable have also been proposed by Clingerman et al. (2007), who assumed that the decrease in activity in response to stressors was a behaviour aimed at minimising the energy expenditure associated with swimming. In the second growth phase, the larvae showed positive allometric growth in terms of head parameters. This late development could reflect insufficient development of their respiratory structures, their swimming capacity, and their capacity to digest prey, particularly in the moderate group at 8.5°C. This behaviour could also be related to jerky swimming behaviour and low anxiety (Jonsson and Jonsson, 2009). In conclusion, the moderate group appeared to be intermediate with respect to the two profiles proposed by the POLS theory, with differences in aggressive behaviour, parasympathetic system activation, and metabolism (table 3).

The heavy group showed a different profile from the control and moderate groups, with high efficiency of yolk sac use and reductions in hatch size that may be correlated with a high metabolism. The small size at hatching could reduce foraging efficiency and swimming performance and could be related to lower intraspecific competitiveness, predator escape capacity, and reduced fitness (Kamler, 2002) . There was a strong increase in AChE activity in the heavy group at 8.5°C, which could be linked to the results of our analysis of behaviour and oxidative stress, which showed more stressed larvae with jerky swimming behaviour. This hyperactive behaviour has previously been observed in many species contaminated with PCB and was suggested to be caused by alterations in the dopaminergic system. This is also supported by a delay in visual development noted during the first phase of growth and a delay in the growth of the myotome, which may be related to jerky swimming behaviours (Jonsson and Jonsson, 2009) and low anxiety. This observation was also described by Gandar (2015), who reported altered swimming behaviour and poor cohesion among individuals. This increase in activity may be related to the high yolk sac efficiency that supports this hyperactivity. In addition, an increase in oxidative stress also leads to an increase in metabolic cost (Gandar, 2015), which could lead these larvae to experience decreased growth, reflected by their smaller size compared with other conditions. However, during the second growth phase, the time between mixed and exogenous feedings was smaller at 8.5°C in the heavy group, which could allow the larvae to avoid the moment of point-of-no-return and allow compensatory growth, increasing their chance of survival as observed in other species (Trabelsi et al., 2012). This hypothesis appears to be supported by the fact that larvae incubated at 8.5°C in the heavy group showed the same size in the exogenous feeding stage as their non-contaminated congeners incubated at 8.5°C. In the juvenile stage, the heavy group was larger than their conspecifics, and many authors have hypothesised that hormonal dysfunction could affect growth given the endocrine-disrupting nature of PCB (Schnitzler et al., 2008). In conclusion, the heavy group tended to have high metabolic rates, as described by Réale et al. (2010) or could be considered proactive with a high growth rate and high levels of aggressiveness and activity, in addition to an increase in oxidative stress, which would certainly generate an increase in metabolic cost and hence high efficiency of yolk sac use (table 3).

The results of the three profiles did not perfectly agree with the POLS theory; however, a recent study highlighted some caveats to the theory put forward by Reals et al. (2010) and suggested that it is highly context-dependent (Montiglio et al., 2018). Thus, our study experimentally supports a hypothesis put forward by Hämäläinen et al. (2020) that POLS should be stronger in unfavourable environments. Indeed, Alfonso et al. (2018) demonstrated that inter-individual differences are interconnected from a physiological and behavioural perspective, resulting in a continuum between two extreme types of individuals (proactive and reactive).

4.6 Conclusions

The present study of a single fish population describes for the first time the potentiating effects of PCB contamination and increases in water temperature on Arctic char at different life stages. Both stressors acted synergistically on survival, yolk sac efficiency, and AChE levels. Temperature alone could influence the development time and aggressive behaviour, whereas PCB contamination alone affected activity. These interactions depend on the larval development stage. According to Hämäläinen et al. (2020), modifications of these life traits could have repercussions throughout the life of fish due to irreversible developmental plasticity in early life. Based on the POLS theory, the results emphasised a continuum of variation, including possible physiological and behavioural variation, as a function of PCB contamination and an increase in temperature.

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Ethical considerations

This study was carried out in strict accordance with recommendations contained in the Guide for the Care and Use of Laboratory Animals and French legislation (agreement number: E74 300-4). The study protocol was approved by the Committee on the Ethics of Animal Experiments of the CECCAPP (Directive 499 2010/63/EU). All efforts were made to minimise animal suffering.

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FIGURE LEGENDS

Figure 1: Morphometric characters measured among Arctic char fry from hatching to the juvenile stage. ED, eye diameter; HH, head height; HL, head length; JL, jaw length; TL, total length; TaiL, tail length; CaH, caudal fin height; MH, myotome height (modified from Trabeslki et al., (2013)).

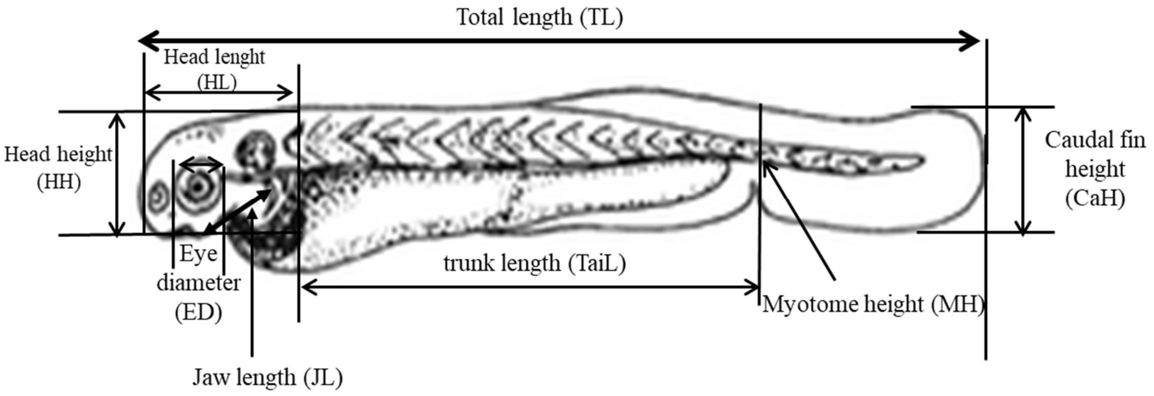

Figure 2: Temporal evolution of the percentage of survival for each condition. Dark red corresponds to the conditioning of larvae incubated at 8.5°C and contaminated with 250 ng/g of maternal polychlorinated biphenyls (PCB). The red line corresponds to the conditioning of larvae incubated at 8.5°C and contaminated with 125 ng/g of maternal PCBs. The yellow line corresponds to the conditioning of larvae incubated at 8.5°C and contaminated with 50 ng/g of maternal PCB. The dark blue line corresponds to the conditioning of larvae incubated at 4°C and contaminated with 250 ng/g of maternal PCBs. The blue line corresponds to the conditioning of larvae incubated at 4°C and contaminated with 125 ng/g of maternal PCB. Sky blue line corresponds to the conditioning of larvae incubated at 4°C and contaminated with 50 ng/g of maternal PCBs.

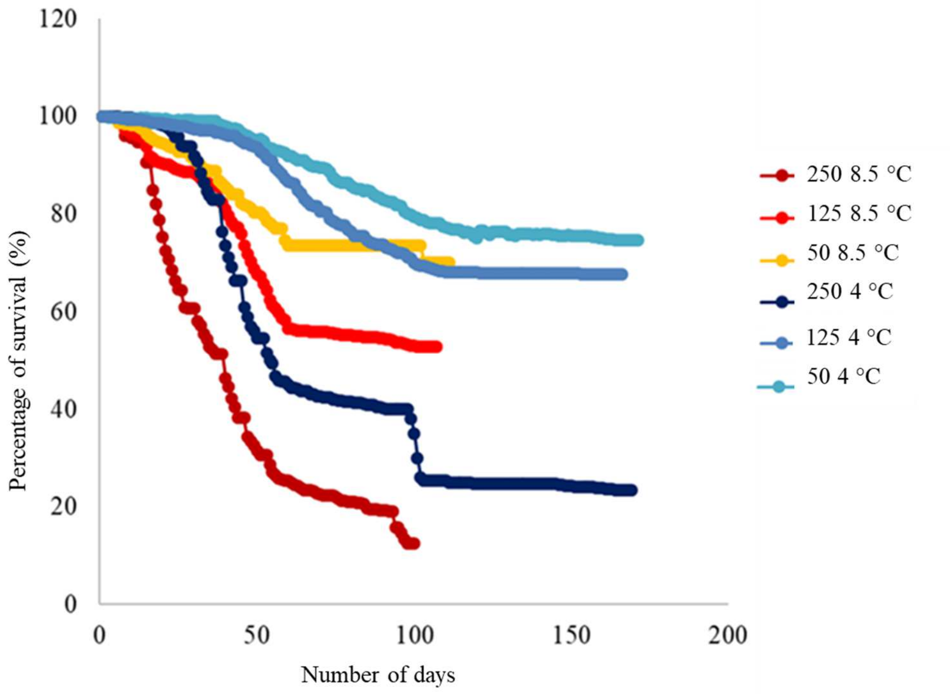

Figure 3: Summary of the different behavioural parameters tested. 1) Distribution of contact as a function of temperature. Data are expressed as the mean ± standard error. Stars indicate statistically significant differences (χ^2 test $p < 0.05$). 2) Number of physical contacts (mean = 6) between larvae in juvenile stage 1 as a function of temperature. The black line represents the median. Stars indicate significant differences between temperatures ($p < 0.05$) assessed using a post hoc Student *t* test. 3) Graph representing the overall activity time and activity typology in seconds for each PCB contamination group. Data are expressed as the mean ± standard error. Letters represent statistically significant differences ($p < 0.05$) obtained using the Student *t* test.

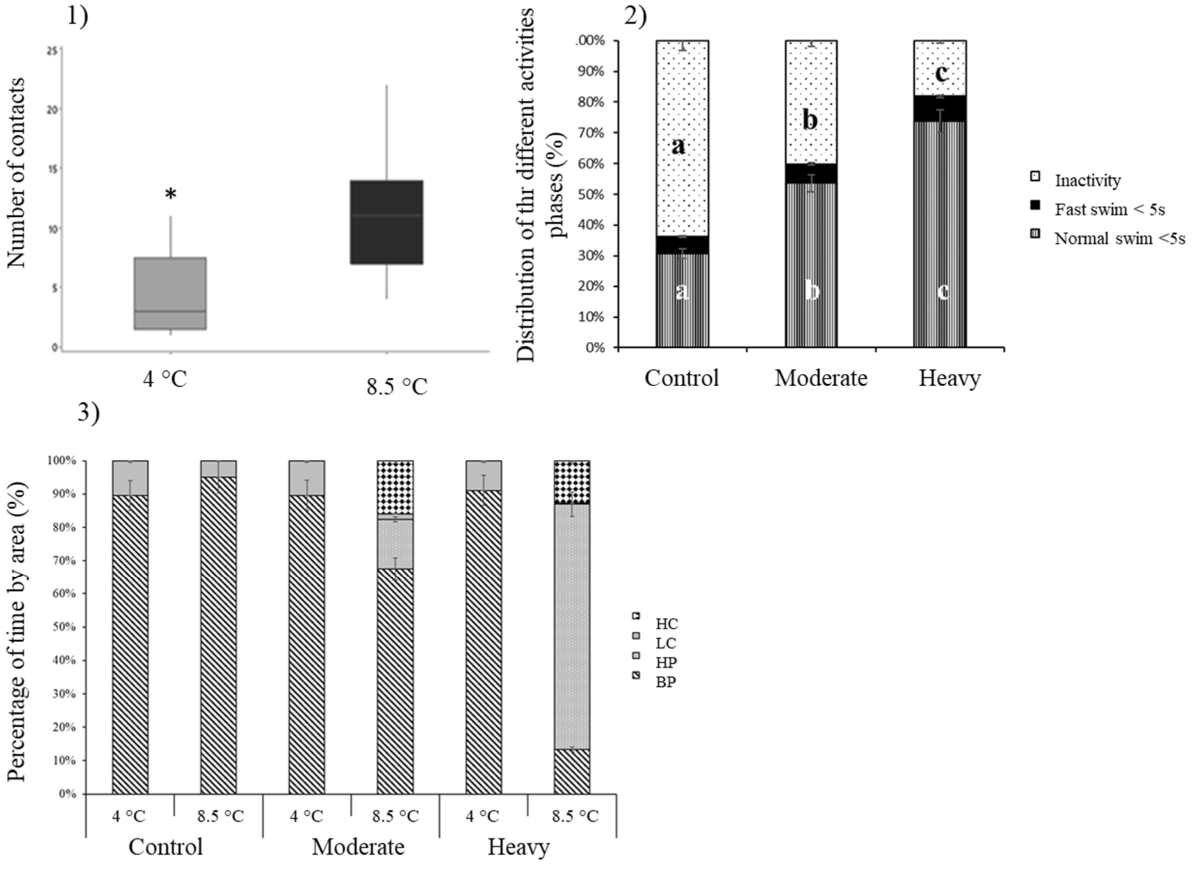

TABLE LEGENDS

Table 1: Experimental design for female fish that received a single intraperitoneal injection of PCB (Aroclor 1254) one month before egg laying.

Groups	Females injection	Oocytes contaminations	Muscle contaminations
Control	0 ng / g	50 ± 5 ng / g	7.6 ± 0.2 ng/g
Moderate	500 ng / g	102 ± 11 ng /g	48.6 ± 7.4 ng/g
Heavy	1 000 ng /g	239 ± 10 ng / g	78.9 ± 6.1 ng/g

Table 2: Summary table of physiological parameters tested. The data are expressed as the mean ± standard error. Letters represent statistically significant differences ($p < 0.05$) obtained using the Student t test. H: hatching; Emg: emergence, MF: mix feeding; EF: exogenous feeding; J: juvenile.

Biodemographic	Survival		Blastula stage		Malformation rate		Specific Growth Rate	
	F	P	F	P	F	P	F	P
Temperature (T°C)	0.01	0.92	12.74	***<0.01***	0.66	0.42	3.79	0.05
PCB	5.55	***<0.01***	127.9	***<0.01***	39.31	***<0.01***	1.14	0.28
Stade	34.38	***<0.01***			0.19	0.94	43.87	***<0.01***
T°C & PCB	0.96	0.38	46.71	***<0.01***	17.83	***<0.01***	9.23	***<0.01***
T°C & Stage	0.06	0.81			0.91	0.46	75.21	***<0.01***
Stage & PCB	3.60	***<0.04***			1.93	0.11	27.39	***<0.01***
T°C & Stage & PCB	0.94	0.393			3.77	***<0.01***	42.75	***<0.01***
Behavioral	Activity		Aggressiveness					
	F	p	F	P				
Temperature (T°C)	0.15	0.70	4.66	***<0.04***				
PCB	6.84	***<0.01***	0.73	0.50				
T°C & PCB	0.53	0.46	0.41	0.67				
Physiological	EUV		SOD		CAT		AChE	
	F	p	F	P	F	p	F	p
Temperature (T°C)	23.55	***<0.01***	0.71	0.4	0.44	0.50	12.0	***<0.001***
PCB	34.33	***<0.01***	5.69	***<0.01***	0.51	0.47	7.54	***<0.01***
Stage	19.18	***<0.01***	0.49	0.48	58.84	***<0.01***	5.38	0.05
T°C & PCB	13.69	***<0.01***	2.41	0.09	4.99	***<0.01***	5.59	***<0.01***
T°C & Stage	6.36	***<0.01***	0.02	0.89	0.41	0.74	4.80	0.05
Stage & PCB	13.32	***<0.01***	5.64	***<0.01***	0.2	0.89	6.98	***<0.01***
T°C & Stage & PCB	5.59	***<0.01***	3.27	***<0.04***	0.7	0.97	3.88	***<0.04***

Table 3: Summary of responses to the parameters responding to the POLS theory (Réals et al. 2007); 3 and black colour: strong response, 2 and dark gray colour: intermediate response, 1 and light gray colour: weak response.

	Temperature (°C)	4 °C			8.5 °C			Theory	
	Oocytes PCB (ng/g)	Control	Moderate	Heavy	Control	Moderate	Heavy	Slow / Fast	Rapid / Proactifs
Bio-demographic	Growth rate	1	2	3	1	2	3	1	3
	Allometric growth	1	1	3	1	1	3	1	3
Behaviour	Aggressiveness	1	1	1	3	3	3	1	3
	Activity	1	3	3	1	3	3	1	3
	Anxiousness	1	1	3	1	1	3	1	3
Physiology	Oxydatif stress (SOD. CAT)	1	3	3	1	3	3	1	3
	AchE	1	2	3	1	2	3	3	1
	Metabolism = Yolk sac efficiency	3	1	3	3	1	3	1	3
Total scoring		10	14	19	12	17	24	10	19