Master Thesis
MSc Ecology and Evolution

Pace-of-life syndrome in three-spined sticklebacks *(Gasterosteus aculeatus)*: integrating life-history strategies and behaviour across climatic conditions.

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Abstract

In virtually every animal population, individuals vary through a wide range of personality traits and life-history strategies. According to the pace-of-life (POL) hypothesis, behavioural and life-history traits should covary following a continuous gradient with at the most extremes a slow and fast POL. Evidence on such a syndrome is however currently insufficient, with very mixed research findings. In this study, I assess the validity of the POL syndrome hypothesis in a migratory three-spined stickleback population, using a combination of datasets collected both in the wild and in semi-natural laboratory conditions. I also evaluate the influence of ecological conditions on the presence and strength of the covariation between traits across years, and therefore on the POL syndrome. I focus on the following characteristics: life-history strategies: (1) migration timing, (2) fish size or condition; behavioural traits: (3) boldness, (4) dispersal and (5) exploratory behaviour.

Although life-history traits were correlated as expected, I did not find that life-history strategies and behavioural traits covaried in line with the POL syndrome hypothesis. I also found that the trait covariation varied significantly throughout the years, which could only partly be explained by differences in water temperatures. I conclude that if the POL syndrome does occur, its structure is likely to be much more complex than previously assumed and studies on the relationship between life-histories, physiologies and behaviours over various gradients of ecological conditions will therefore be needed in order to fully evaluate the validity of the POL hypothesis in natural populations.

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Introduction

In virtually every animal population, individuals vary in behaviors. When these differences in behaviour are consistent over time and across different situations, they are defined as personalities (Gosling, 2001; Sih *et al.,* 2004; Réale *et al.,* 2007). Animal personality traits can therefore be defined as behavioral tendencies that vary consistently across the members of a given population, across situations, contexts and time (Biro & Stamps*,* 2008). Because of their consistency, personality traits are valuable for ecological and evolutionary studies. For example, they can be indicators of the condition and quality of a certain habitat (Sih et al., 2004; Réale et al., 2007). Riechert & Hall (2000) found that different habitat types lead to differences in anti-predator behaviour and aggressiveness in individual desert spiders (*Agelenopsis aperta).* Indeed, they found that spiders are aggressive towards both conspecifics and prey and bold towards predators in habitats that are resource-limited, arid and highly competitive, but that they are shy and non-aggressive in food-rich, riparian, non-competitive habitats (Riechert & Hall, 2000). Personality traits can explain apparent maladaptive behaviours (Sih et al., 2004; Réale et al., 2007). Indeed, in many instances, authors have suggested that maladaptive behaviours appear as a result of a spill-over of an important general behavioural tendency that occurred in other contexts or situations (Sih *et al.,* 2004). For example, Jamieson (1986) noted that host feeding of a brood parasite, regardless of its resemblance to the host's own offspring, could be due to the strong selection favoring parental feeding of their own offspring. Another example can be seen in fishing spiders (Dolomedes spp., Pisauridae: Araneae), where it was suggested that excessive sexual cannibalism could be due to a general feeding aggression syndrome expressed over a lifetime. Highly voracious juveniles would grow faster and larger into more fecund females, then continue to be voracious into adulthood and therefore further enhance their fecundity. If there are positive correlations between attack tendencies on prey, including conspecific males, then it could explain the excessive sexual cannibalism (Arnqvist & Henriksson, 1997).

Personality traits between individuals are often correlated, therefore forming behavioural 'syndromes', where distinct behaviours are evidently not independent from each other (Sih *et al*., 2004; Dall *et al.* 2004; Sih & Bell, 2008).

There is also growing evidence that the amount and structure of within-population behavioural variation can considerably influence key ecological and evolutionary processes, such as population density and productivity, stability, resilience, and persistence of populations as well as species interactions (Wolf and Weissing, 2012). Additionally, animal personalities are increasingly recognised as an important factor affecting intraspecific and interspecific competition, as well as the structure and dynamics of ecological networks (Whitham *et al.,* 2006; Bolnick *et al.,* 2003 & 2011; Hughes *et al*., 2008; Violle *et al.,* 2012). The implications of personality differences for ecology and evolution are therefore numerous.

Another important implication of personalities is the potential impact it has on the life-histories of individuals (Wolf and Weissing, 2012). Differences in behavioural types are indeed expected to be associated with differences in the mortality and fecundity profile of individuals, and therefore strongly impact an individual's fitness (Biro & Stamps*.,* 2008; Réale *et al.,* 2010; Smith and

Blumstein, 2008). This is due to three main reasons. Firstly, some personality traits are related to mortality risk and fecundity. This is the case for boldness, aggressiveness, activity and dispersal tendencies, for example (Réale et al., 2010). Secondly, it is common that different behavioural types be consistently found in different habitats, therefore facing different competition, predation or parasitic conditions, all of which affect fitness (Nowak *et al.,* 2010; Duckworth, 2006). Thirdly, personalities are linked to other morphological or physiological characteristics, as part of a pace-of-life syndrome, which again reinforces mortality and fecundity differences (Wolf *et al.,* 2007; Conrad *et al.,* 2011*;* Réale et al., 2010).

The pace-of-life syndrome hypothesis (hereafter POL) is a conceptual model that integrates variation in life history, physiology and behaviour (Réale *et al.,* 2010). It first stated that closely related species should differ in a suite of physiological traits that have evolved with each species' life-history particularities (Ricklefs & Wikelski 2002; Wikelski et al 2003; Martin et al 2006). Although first developed on the species level, this concept was later applied to the individual level, thus when consistent individual variation was present within a same population (Réale *et al.,* 2010).

The POL syndrome distinguishes individuals by their tendencies and characteristics, following a continuous gradient with at the most extremes a slow and fast POL (see: Appendix A). In line with this theory, individuals typically gravitate towards either side of the spectrum: individuals with a 'slow' pace-of-life are expected to exhibit relatively slow growth rates, long life spans and to reach sexual maturity later in life. These life-history strategies are generally correlated with both physiological responses, such as slower metabolic rates and stress reactivity but higher immune responses, and behavioral responses, such as low aggressiveness and high sociability (Réale et al., 2010). On the contrary, individuals with a fast pace-of-life are predicted to be less risk averse due to higher metabolic rates, therefore being more aggressive and less social (Réale et al., 2010). Following this hypothesis, it would therefore be possible to predict a number of an individual's traits by knowing about a few of its other traits. For example, a highly social, non-aggressive shy individual is expected to also have a relatively low growth rate, and live a longer life than an individual with opposite behavioural traits.

Although the pace-of-life concept is an appealing idea, current empirical evidence is mixed. Indeed, some research found evidence supporting the pace-of-life syndrome hypothesis, such as Niemelä *et al.* (2013) did in their study on western stutter-trilling cricket (Gryllus integer). Oppositely, Závorka *et al.* (2015) carried out research on trout stream *(Salmo trutta)*, and found no correlation between laboratory activity (where behaviour was assessed) and growth rate or survival, therefore going against the POL hypothesis. A meta-analysis carried out by Royauté *et al. (*2018) found little support for the general predictions of the pace- of-life syndrome hypothesis. Indeed, they concluded that, based on current research, behavior did not seem to be integrated with physiology or life-history in a way that is consistent with the POL syndrome hypothesis. Some argue that part of this disagreement is because many studies testing the pace-of-life syndrome only focus on the relationship between one personality trait and one single life-history trait (Bijleved *et al.,* 2014; Biro & Stamps*,* 2008; Cole and Quinn, 2014). Because personality and life-history traits are fundamentally multidimensional, it is argued that reliable assessment of this relationship should therefore be based on multidimensional

measures (Monceau *et al.,* 2017). More comprehensive research is therefore needed in order to better assess the validity of this hypothesis.

A suitable setting in which to study this hypothesis is within migrating individuals. These individuals make the initial choice of migration, that is they choose to move from one region to another, and then later disperse to a breeding site located at more or less greater distances. Upon migration into a new habitat, dispersal can be crucial to overcome competition and secure a high quality territory. Dispersal implicates three successive behavioural stages: departure from a current patch, movement between patches (transience) and settlement in a new patch (Bowler & Benton 2005; Clobert et al. 2009). Highly dispersive individuals will be favored when close-by areas are low quality and unsuitable, but this advantage will not remain true if dispersal to a further patch is not synonymous to higher habitat quality. This is because high dispersal is thought to be costly, as it can be risky and involve a substantial energy investment. The integration of certain traits that enhance an individual's success of dispersal and settlement to a new and further habitat, such as boldness and high aggressiveness, can counterbalance these costs and lead to the maintenance of diverse dispersal strategies within a species (Duckworth, 2008; Aberts and Altmann, 1995; Gandon and Michalakis, 1999; Ravigné *et al.,* 2004).

Variations in both migratory and dispersal strategies are often documented within a population, as some individuals may choose to migrate and/or disperse more or less early and quickly, which in turn is also reflected in their morphology and physiology. This was for example shown to be the case for migration in sockeye salmon *(Oncorhynchus nerka)*, where timing and duration of juvenile migrations were negatively correlated with both life‑history strategies and body size (Hanson *et al.,* 2008). Similar results were found for dispersal, where research has shown that in many cases, body size is positively correlated with dispersal (Sutherland *et al.,* 2000; Paradis *et al.*, 1998). Both within and between populations, individuals are therefore expected to have different suites of traits depending on their migration strategies as well as their dispersal strategies, and could therefore fall under different sides of the pace-of-life syndrome spectrum. Finally, the degree of variation of both migration and/or dispersal strategies is likely to depend on the environmental conditions. For example, it is probable that in cold years, where food sources are likely to be scarce, migrating early poses no real advantage, whereas in warmer years, growing fast and migrating early could be advantageous as early individuals would gain access to key resources before their slower counterparts. This would be reflected in the strength of POL syndrome observed, with cold years therefore resulting in an absent or less strong POL syndrome, and warm years leading to stronger correlations and a more pronounced POL syndrome.

Here, I tested whether the pace-of-life syndrome could be detected in populations of anadromous three-spined sticklebacks (*Gasterosteus aculeatus*). These small fish are an increasingly common model species due to their small size, robustness, large behavioral repertoire and adaptability. They are widely distributed in the northern hemisphere, inhabiting coastal marine waters, brackish waters and various freshwater habitats, and usually migrate from march up to July from marine or estuarine environments, to spawn in streams (Bell & Foster, 1994; Ostlund-Nilsson *et al.,* 2006). Three spined sticklebacks therefore face two types

of movements through the course of their adult lives: migration from sea to freshwater, and later dispersal within freshwater to secure a viable breeding ground (Ostlund-Nilsson et al., 2006). Because of their broad geographical and ecological distribution, sticklebacks show an exceptional range of phenotypic diversity (Bell & Foster, 1994). This diversity is also seen in the great variety of behaviours they display. In fact, stickleback populations have been shown to differ profoundly in behavioral types (Herczeg et al., 2009; Ramesh *et al.,* 2021), perhaps reflecting the differences in selection pressures they are subjected to, such as predation risk (Bell, 2004;2005).

The aim of this study is therefore to investigate the validity of the pace-of-life syndrome concept in a migratory three-spined stickleback population, using a combination of datasets collected both in the wild in different ecological conditions and in semi-natural laboratory conditions. To do this, I quantified the among-individual phenotypic variation present within the same population and tested if these traits formed a POL syndrome. I tested if trait means and trait correlations varied across the years, specifically with changes in climatic conditions.

Several predictions were made regarding the link between migration timing, individual size and behaviour and degree of dispersal upon arrival. If we are to follow the POL hypothesis proposed by Réale *et al.,* (2010) (Appendix A) and apply it to this research, the traits studied would therefore ideally form the following schematic:
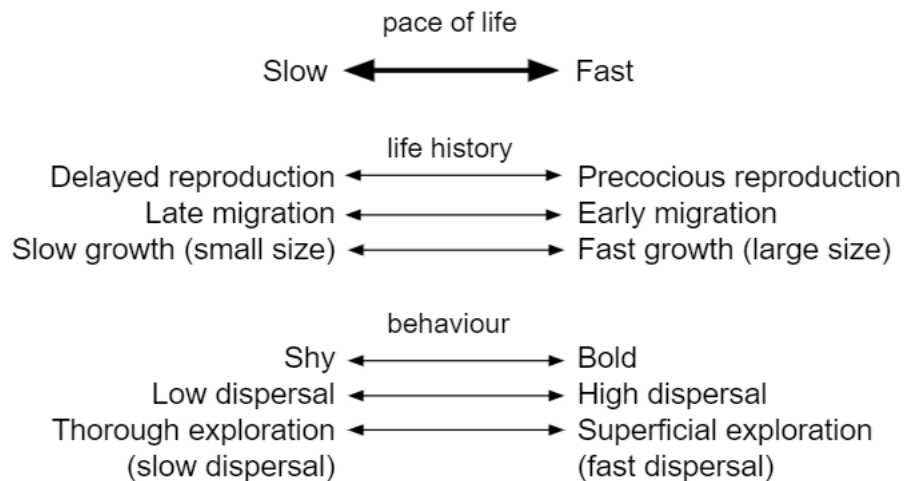
Figure 1: Schematic of the potential integration of different traits along a pace-of-life continuum. Double arrows illustrate presumed continuous variation in life-history strategies among individuals in a population, and its assumed relationship with personality and physiological traits (based on schematic by Réale et al., (2010) and adapted to the traits of this study).

Although Réale *et al.,* (2010) did not mention migration, size and speed of dispersal specifically, they do state that a precocious reproduction, high growth rate, and superficial exploration can all be used as indicators of the pace of life of an individual. Considering sticklebacks spawn in freshwater environments, reached only after migration from sea, an early migration would also most likely lead to a precocious reproduction relative to later individuals. In the same way,

individuals with high growth rates would be bigger upon arrival in freshwater environments. Finally, a fast dispersing individual is also likely to be a more superficial explorer than a slower dispersing individual, as it would overall spend less time in each patch before moving on to the next one. Based on the POL syndrome, I expected individuals with a 'fast' pace of life would exhibit faster growth rates (thus reaching larger size at sexual maturity), be bolder (or more likely to take risks, evaluated here through exploratory behaviour in an unknown environment), migrate earlier in the season, and disperse further than individuals with a 'slow' pace of life. These 'slow' individuals would in turn exhibit slower growth rates (thus be smaller at sexual maturity), be shier, migrate later and disperse less. All of this was also expected to be influenced by climatic conditions, where the POL syndrome would be tighter in warmer years, and I therefore expected correlations to be stronger in warm years. This is because I expect that competition would be higher in years where food resources are more abundant and where arriving early and securing territories harbouring these resources is therefore highly beneficial. Oppositely, in colder years where conditions are harsh and resources are likely to be scarce, arriving early would pose less of an advantage and the POL syndrome hypothesis would therefore be less pronounced. In order to investigate this, stickleback counts under different climatic conditions will be analysed, as well as the influence of temperature on stickleback size and timing of arrival.

Methods

Data collection

Several different datasets were used, collected in different ways over several years:

Table 1: All data sets used for this study, with years and contents of each set.

Data set	Years recorded	Content
Monitoring data	2014, 2015, 2017, 2018, 2019 & 2020	Count of fish caught between March and May (+ June in 2020) & sampling duration
PIT tagging data	2017, 2019 & 2021	Individual PIT number of fish caught, catch and release date, length and weight of each fish
Dispersal data	2017, 2019 & 2021	Individual PIT number of fish detected date of detection and coordinates of detection location
Pond data	2020 & 2021	Behavioural data of fish tested, weight, standard length and plate morphology
Temperature data	Jan 2013 to April 2021	Average monthly temperatures at Nieuwe Statenzijl

Monitoring, PIT tagging and temperature data

Monitoring of fish was carried out using crossnets located at the Nieuwe Statenzijl and fyke nets located at the Hongerige Wolf pumping station in the north of the Netherlands, close to the German border (see Fig. 2). This was done in 2014, 2015, 2017, 2018, 2019 and 2020 during the migration period of sticklebacks (February till May) (Table 1). The nets used measured 1m by 1m, were funnel-shaped and were approximately 100cm deep with a mesh width of 1mm. They allowed for a part of the water column to be sampled vertically. Cross nets were collected every ten minutes, for six or seven hours (from low tide to high tide) each sampling day. Nets were therefore checked approximately 36 times within one sampling session. Variables measured were fish quantity, size, weight, as well as morphologie (type of plating) when possible.

During three of the monitoring years, in 2017, 2019 and 2021, fish were also professionally individually tagged by implanting a passive integrated transponder-tag (PIT-tag, 13mm) into their stomach cavity under anaesthesia. The PIT-tag provided all fish with a unique identifier, and in the event that an individual stickleback was recorded at an antenna, it automatically provided the date and time of detection. This was done so further experiments could be carried out, such as dispersal analyses and behavioural tests. Water temperature was also recorded daily in both Nieuwe Statenzijl and Termunterzijl, from January 2013 to April 2021.

Figure 2: Left: Monitoring of stickleback population at the Nieuwe Statenzijl using cross nets; Right: Monitoring of stickleback population at the Hongerige Wolf pumping station, using fyke nets (April 2021, personal archive)

Dispersal data

Following the POL hypothesis, bigger and bolder fish were expected to be more dispersive than later, smaller and shier individuals. Boldness was established to be higher for individuals who showed higher exploratory behaviour in unknown environments.

These expectations were tested in two instances: first, in a laboratory set-up where fish behaviour was assessed thanks to interconnected ponds, and then in the wild where dispersal was recorded via several antennas.

In the wild

In the wild, fish dispersal was assessed using PIT tagging technology, in 2017, 2019 and 2021. Sticklebacks were caught either in Nieuwe Statenzijl or Hongerige Wolf, and were released accordingly. They were then able to disperse in-land. Four different locations were equipped with antennas able to detect the PIT tags: Hongerige Wolf (5km from the coast), Ulsda (9km from coast), De Bult (10km from coast) and Veelerveen (20km from coast) (See Appendix B). This enabled the collection of data on where and when fish were detected, therefore showing both how fast and how far individuals dispersed.

In the pond experiments

Study site and field collection

Fish were captured in 2020 and 2021, using cross nets (1m by 1m), fyke nets, as well as large seine nets dragged by two boats in order to sweep the whole sampling area (see Fig. 3). They were captured during their migration, from March to April, at two close-by locations. The first was at the sea lock of Nieuwe Statenzijl in the Dollard estuary (n=244 individuals), and the second was further down the waterway in front of the Hongerige Wolf pumping station located in a side arm of the Westerwoldse Aa river (n=16 individuals). Fish were therefore captured both at the mouth of the river and after a divergence from the main waterway. This was done in order to prevent any bias in our sample of the population (Álvarez-Quintero *et al.,* 2021). After being tagged, fish were transported to the laboratory of the university of Groningen in aerated containers. All individuals were also weighed, measured (both total and standard length) and plate morphology was recorded (either low, semi or completely plated), although these morphology data were not used in present analyses.

Figure 3: Monitoring of sticklebacks at the sea lock of Nieuwe Statenzijl using seine nets (April 2021, personal archive)

The laboratory set-up

The outdoor laboratory is located in Groningen, at the Zernike campus. It is equipped with a mesh ceiling and walls in order to be protected from predators (see Fig. 4). The set-up consisted of 10 connected ponds (dim. Ø163 cm and a height of 102 cm) connected via tubes and supplied by freshwater pumped directly from the waterway adjacent to the area, creating a linear flow in the system of connected ponds similar to those typically experienced in the canals and ditches (flow speed < 0.7 cm/s). This helped to create a cue for migration-like movement. Upon arrival at the laboratory, the fish's standard length was individually measured, before being placed in two groups of 20 in ponds 1 and 6. They were then fed bloodworms and were left to acclimate overnight before any testing occurred (see Appendices C & D for the experimental set-up).

In 2021

The morning after capture, exploratory-migratory tendencies were scored in an experiment set-up consisting of two sets of 5 pond-networks connected via corridors (Ø110 mm, length of 1.5m). Corridors were equipped with antennas at the entrance and exit to record crossings of PIT tagged fish (Fig. 4). Fish were tested in groups of 20 (total n=64) (see Appendix D for a detailed scheme of the set-up). They were confined in pond nr 1 of each pond-network overnight to acclimatise. At the start of the experiment, the corridors were opened and fish were given 4 hours to explore, before being captured again. All data was saved in the PIT-readers connected to each antenna and placed in waterproof chests by the ponds. The fish were then

captured again and were released on the site of capture in the afternoon. Migratory/exploratory tendencies were quantified by the number of ponds visited, the total number of crosses and the latency to reach the last pond. Pilot data had shown that these traits were repeatable.

In 2020

Behavioural tests were conducted in a similar manner in 2020, but a few variations are to be noted. Fish were tested in groups of 10 and were given 24 hours to acclimate prior to the start of the experiment (total n= 196). On the morning of testing (~10 a.m.) the test groups were released into the first pond in the same row of five connected ponds that was used in 2021. All connecting tubes were fitted with circular PIT antennas around the entrance and exit of each pond to record movement of fish between ponds. The connecting tubes were closed off during the first 5 hours of the experiment, so that fish were confined to the first pond where other experiments were taking place using circular PIT antennas (data not used for this project). Five hours after the start of the experiment the PIT antennas were removed from the first pond. At this point, the pump connected to a nearby waterbody was started. Fish were given 30 minutes to recover from the disturbance caused by removing the PIT antennas, after which the connection from pond 1 to the other ponds was gently opened. The movement of fish between ponds was then recorded for the next 16.5h (~3.30 p.m. ~8 a.m.). Only the data from the first 4 hours was used for this research project, in order to be in keeping with the experiments carried out in 2021.

Figure 4: Left: Experimental pond set-up in the University of Groningen; Right: Antennas on each end of the tube connecting the ponds (Personal archive, March 2021)

Statistical Analyses

All statistical analyses and figures were performed using the R software (version 4.0.2).
The main packages used were Tidyverse (Wickham et al., 2019), multcomp (Hothorn *et al.,* 2008*)*, car (Fox & Weisberg, 2019), lme4 (Bates *et al.,* 2015), ggpubr (Kassambara, 2020), and lme4 (Bates *et al.,* 2015).

Temperature data

First, a correlation analysis was conducted on the water temperatures in Nieuwe Statenzijl and Termunterzijl. These data were provided by the Waterschap Hunze en Aas, who recorded monthly water temperatures from 2000 to 2021. As the temperatures from both sites were strongly correlated, and in order to facilitate future work, all analyses were based on the temperatures recorded in Nieuwe Statenzijl.

Then, in order to more accurately account for the impact of climatic conditions in statistical modeling, I ran a climate window analysis using the *climwin* package (Bailey & Van De Pol, 2016; van de Pol et al., 2016). This helped detect which time period, or 'window' over which my variables were sensitive to the environment. In other words, to see which months' water temperatures were more likely to have an influence on the different traits measured, instead of choosing an arbitrary time-span. I restricted the possible time window by letting the starting date vary for up to 4 months prior to the start of the count. The Climwin package compares the model outputs of thousands of models, the AIC of which it plots graphically as when the temperature window opens (y-axis) and closes (x-axis) in relation to the reference date, which in the case of this study was set to the start of the count of sticklebacks (Samplonius *et al*., 2018).

Count, size and timing analyses

The aim was to investigate how timing of migration, here represented by the count per hour, covaried with fish size (here, fish length) and environmental temperatures, in order to assess the validity of the POL syndrome in this population of sticklebacks.

Variation in count (n=5559 ind.), size (n=3316 ind.) and timing (n=5559 ind.) was therefore analysed in relation to water temperature, year and the interaction between year and temperature. In all models, year was fitted as a factor, with 2014 used as reference category.

The first model on count was fitted with a generalized linear model (GLM) with a quasipoisson distribution. Then, models on both size and timing were fitted with a general linear mixed model (GLMM) with a gaussian distribution where year was fitted as a random effect. In all models, it was also examined whether temperature had a quadratic effect, to account for potential non-linear effect. Quadratic terms were only kept in models if significant

Dispersal analyses

In the wild

Using the detection data at PIT antenna placed along the river (see Appendix B), I analysed whether variation in dispersal distance covaried with condition (fish length/weight) and/or timing of arrival using GLMM where years were fitted as random effects (n=106 ind.).

First, the probability that a fish was detected in the wild was analysed, looking at two main variables: their 'condition', calculated as the ratio of length/weight, and their timing of arrival.

This was done using generalized Linear Mixed-Effects Models (GLMER) with a binomial distribution, and with Year as random effect.

If PIT tagged fish were detected, their dispersal distance was then assessed using the geological coordinates of the antennas where fish were recorded, and of the antenna of origin (first release). Again, the influence of fish condition and timing of arrival was statistically analysed using spearman correlation tests

In the pond experiments: quantifying consistent individual migratory tendencies

The antennas used in 2021 were sometimes faulty, which lead to fish being read at antenna 1, 2, 3 and 5 but not 4, for example. In the few instances this occurred, the average time of dispersal was calculated using the data from the other antennas, and a new data point was created to replace the missing one. This was done in order to statistically analyse the data accurately, as missing values could lead to faulty conclusions.

Two main components of dispersal were assessed with these pond experiments: the number of ponds crossed, that reflects dispersal distance in the wild, and the latency to reach the last ponds crossed, to quantify variation in speed of dispersal.

Variation in condition and timing or arrival were analysed in relation to dispersal and latency using GLM with a quasipoisson distribution. The probability of moving depending on fish condition (length/weight) was also analysed using a GLM with binomial distribution.

The same analyses were carried out for latency, with a quasipoisson distribution also (n=260 fish tested in total, and n=47 fish crossed at least one pond).

It is also important to note that repeatability was estimated and it was confirmed that migratory tendency is highly repeatable, based on the data gathered in 2020, as repeatability was found to be 0.86 (95%CI = [0.768, 0.913]).

Results

Climate windows analyses

For all three traits studied, i.e stickleback size, count and timing of arrival, the analyses yielded similar results with little variation. I therefore chose to base my future analyses mainly on the results obtained from the climate window analysis with highest power and resolution: the one ran for the 'count' variable. It showed that the best climate window predicting variation in the studied traits spanned from 4 to 1.5 months prior to the start date. As the start date was the 7th of March 2014, and based on the result of the other climate windows, I thus used the mean temperature over November, December and January of each year as a climate variable. The mean temperature of these 3 months, for each year was as follows: 2014=5.29°C, 2017=3.28°C, 2018=5.3°C, 2019=5.27°C, 2020=6.77°C and 2021=3.7°C. See Appendix E for output of the climate window analysis on stickleback count.

Monitoring, PIT tagging and temperature data

<u>Stickleback count, size and timing of arrival across the years</u>

The data gathered between 2014 and 2020 shows that the number of sticklebacks caught per hour was highest in 2014 and has since then been declining significantly each year, the lowest being in 2020 (overall effect of year: $F_{(4, 5559)}$=6.96,P=4.54e-05; Table 1, Fig. 4). It is also noteworthy that the sample sizes are relatively different, from 44 counts in 2014 to only 12 in 2018 (Fig.4).

Sticklebacks were significantly smaller in 2014 , whereas their size varied very little between the three later years (2017, 2019 and 2021; overall effect year: $F_{(3, 3316)}$=182.8 ,P=<2e-16; Table 1; Fig.5).

Overall, the timing of sticklebacks varied only slightly across the years, except for for 2018, where sticklebacks arrived significantly 0.337 days later, compared to 2014 and the other years (overall effect of year: $F_{(4,5559)}$=1.59 ,P=0.181; Table 1, Fig.6).

Table 1: Summary of the output of the models on the variation in count/hr, size (standard length) and timing of arrival of sticklebacks caught across different years, starting from 2014. Estimates (β) are presented with their 95% confidence intervals and are denoted in bold if significant.

Fixed effects	Count/hr (n=5559)		Size in cm (n=3316)		Timing of arrival (n=5559)	
	β	(95%CI)	β	(95%CI)	β	(95%CI)
Intercept	**2,554**	**(2.291, 2.796)**	5,879	(5.842, 5.915)	3,81	**(3.650, 3.962)**
Year 2017	-0,376	(-0.879, 0.091)	**0,793**	**(0.667, 0.9185)**	-0,033	(-0.302, 0.230)
Year 2018	-0,88	(-1.768, 0.162)	-	-	**0,337**	**(0.0328, 0.6278)**
Year 2019	**-1,599**	**(-2.399, -0.933)**	0,812	(0.737, 0.887)	0,103	(-0.136, 0.340)
Year 2020	**-2,015**	**(-3.564, -0.961)**	-	-	-0,0004	(-0.328, 0.309)
Year 2021	-	-	**0,527**	**(0.353, 0.700)**	-	-

Stickleback count and size across the years
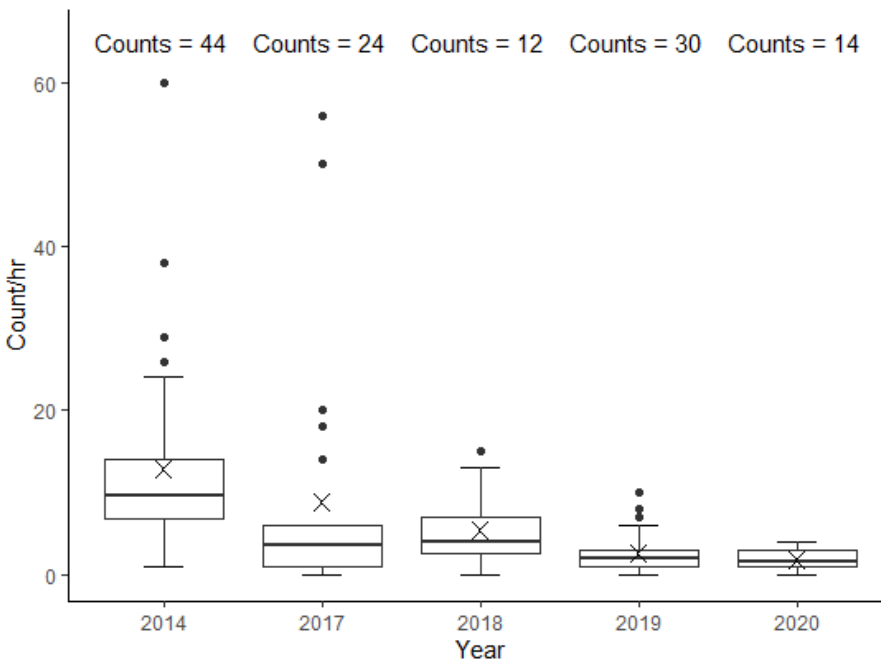
Figure 4: Variation in count of sticklebacks caught between the 2014 and 2020. The crosses show the mean value for each year, and the lines are the median. The error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile. Sample size is shown at the top.
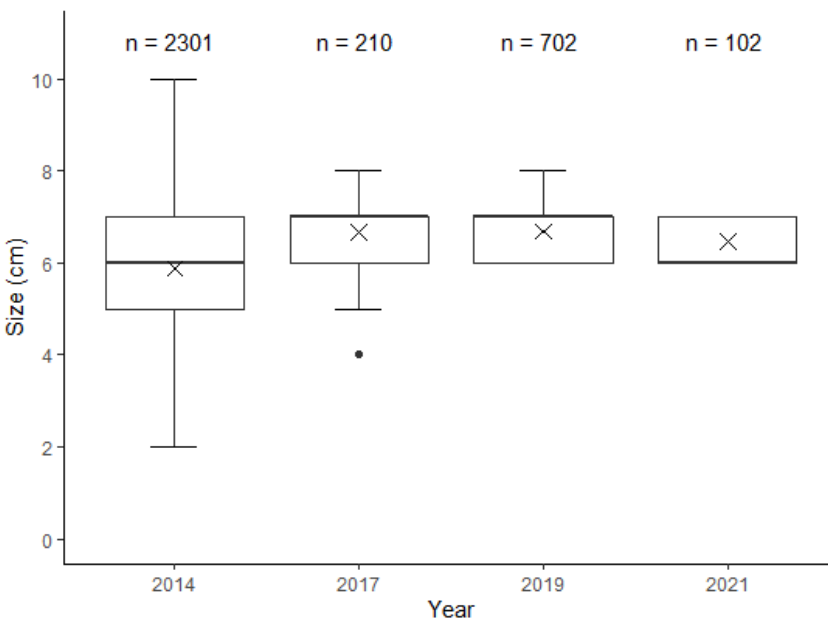
Figure 5: Size of sticklebacks caught each year, in cm. The crosses show the mean value for each year, and the lines are the median. The error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile. ample size is shown at the top.

Timing of arrival of stickleback

Overall, sticklebacks that arrived late were smaller than those that arrived early, as there was a strong negative covariation with size. On average sticklebacks were smaller in all years compared to 2014, as shown by the negative estimates of year (Table 2, Fig. 6). This negative relationship with size was stronger in 2017, with a slope of -6.8 days, and shallower in 2019 and 2021. In 2019, the relationship between timing and size was even slightly positive, but this is probably partly a result of the very narrow range of sizes that year (Table 2, Fig. 7).

Table 2: Generalized Linear Mixed-Effects Model of the date of arrival of sticklebacks of different sizes, for each year. Variance (σ^2) are shown with their associated standard deviation (St. Dev) and estimates of fixed effects (β) with their 95% confidence intervals. Significant effects are denoted in bold. Degrees of freedom (d.f) is also indicated.

Timing of arrival and size (n=3319)			
Fixed effects	**d.f**	**β**	**(95%CI)**
Intercept	3318	**121.32**	**(116.26, 126.38)**
Size	3318	**-10.354**	**(-11.20, -9.505)**
Year 2017	3318	**-65.155**	**(-96.13, -34.18)**
Year 2019	3318	**-116.17**	**(-136.6, -95.73)**
Year 2021	3318	**-80.412**	**(-123.6, -37.20)**
Size xYear 2017	3318	**-6.8000**	**(2.161, 11.44)**
Size xYear 2019	3318	**12.747**	**(9.676, 15.82)**
Size xYear 2021	3318	**8.577**	**(1.853, 15.30)**
Random effect	**σ²**	**St. Dev**	
Year	3.791	1.947	
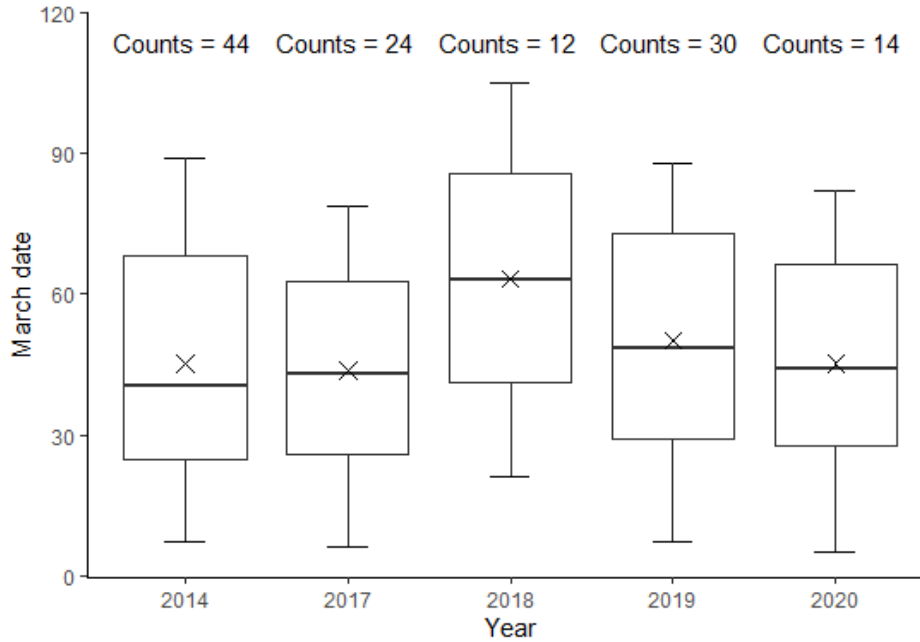
Figure 6: Timing of arrival of sticklebacks caught from 2014 to 2020. The crosses show the mean value for each year, and the lines are the median. The error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile. The number of counts carried out each year is shown at the top.
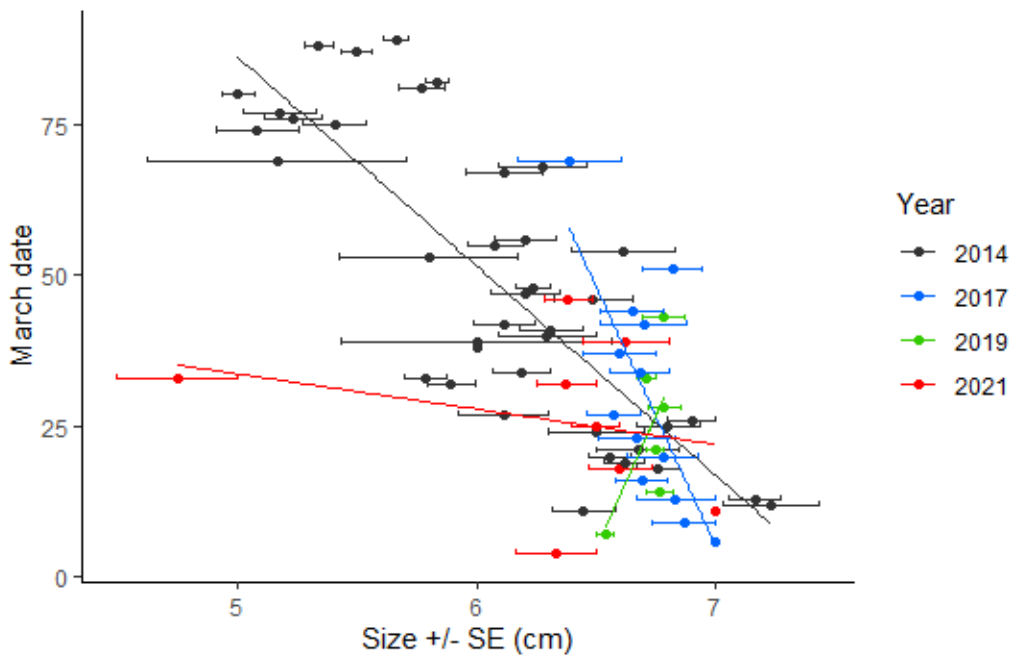

Figure 7: Relations between date of arrival of sticklebacks and size (fish length, in cm) for each year, starting on the first of March. Means are represented with their standard error.

Stickleback count, size and timing for different climatic conditions

The count of sticklebacks caught is shown to depend on the climatic conditions. Results indeed show that there is a significant quadratic relationship, therefore proving that the count of sticklebacks caught per hour is lowest when climatic conditions, here water temperature, tend more towards the extremes (Table 3, Fig. 8). Mild conditions are therefore associated with higher fish counts.

The influence of temperature on the size of sticklebacks was assessed, and results show that size of fish significantly decreased as temperatures increased. Indeed, for each one degree increase in temperatures, fish size decreased by an average of 0.3cm (Table 3, Fig. 9).

The analyses also showed there was no significant influence of temperature on the timing of arrival of sticklebacks (Table 6).

Table 3:General linear model of the count of sticklebacks caught depending on the temperature. 'Temp climate window' is the mean yearly water temperatures of the 3 months prior to the start of catching. 'Temp climate window squared' is the mean yearly water temperature squared, in order to investigate a quadratic relationship. Estimates (β) are presented with their 95% confidence intervals and are denoted in bold if significant. Degrees of freedom (d.f) is also indicated.

Fixed effects	Count/hr (n=3798)			Size (cm) (n=3317)		
	d.f	β	(95%CI)	d.f	β	(95%CI)
Intercept	3797	**-2.659**	**(-3.696, -1.672)**	3316	**7.6000**	**(7.299, 7.901)**
Temp climate window	3797	**2.407**	**(1.951, 2.890)**	3316	**-0.2903**	**(-0.349, -0.232)**
Temp climate window squared	3797	**-0.284**	**(-0.339, -0.233)**	-	-	-
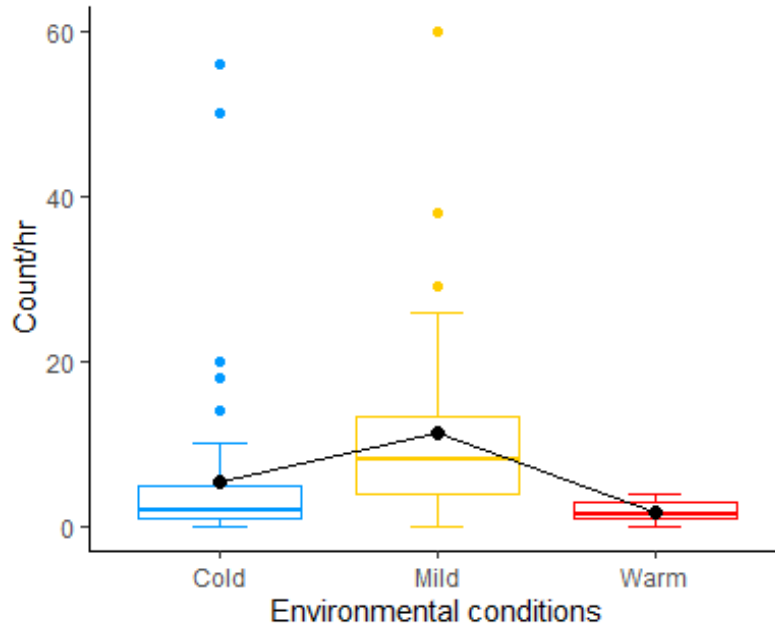
Figure 8: Count of sticklebacks caught for different environmental conditions, here water temperature. The black dots are the means. For graphical purposes, water temperatures were divided into three categories: Cold [1.6°C,4°C], Mild (4°C,6.7°C] and Warm (6.7°C,9.7°C]. Data were recorded in 2014, 2017, 2018, 2019 and 2021.
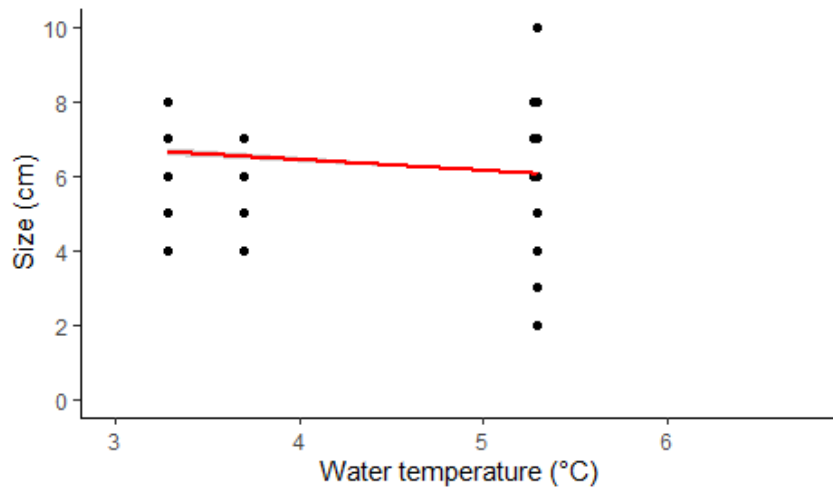
Size of sticklebacks caught for different conditions

Figure 9: Relationship between size (fish length, in cm) of sticklebacks caught and climatic conditions, here water temperature. Data were recorded in 2014, 2017, 2019 and 2021.

Dispersal data

In the wild

The probability that a fish will disperse tended to be negatively correlated with its condition (length/weight), that is, smaller fish were more likely to disperse than bigger ones (Table 4, Fig. 10).
No significant trend was found between probability of dispersal and timing. Timing of arrival therefore does not seem to influence dispersal choice (Table 4).
Neither condition nor timing were found to have a statistically significant influence on dispersal distance ($r_{(1301)}$ = [0.009], p = [0.921], and $r_{(1301)}$ = [0.065], p = [0.495] respectively) (Table 4).

Table 4: Summary of the output of the GLMERs on the probability for sticklebacks to disperse, in the wild. The fixed effects are condition and march date, and only year is fitted as a random effect.Variance (σ^2) are shown with their associated standard deviation (St. Dev) and estimates of fixed effects (β) with their 95% confidence intervals. Significant effects are denoted in bold. Degrees of freedom (d.f) is also indicated.

	Dispersal and condition			Dispersal and timing of arrival		
Fixed effects	**d.f**	**β**	**(95%CI)**	**d.f**	**β**	**(95%CI)**
Intercept	1301	-1.076	(-2.863., 0.692)	1301	-2.455	**(-4.655, -0.337)**
Condition	1301	-0.064	(-0.132, 0.001)	-	-	-
March date	-	-	-	1301	-0.001	(-0.024, 0.0225)
Random effect	**σ²**	**St. Dev**		**σ²**	**St. Dev**	
Year	0.4967	0.7048		0.528	0.726	
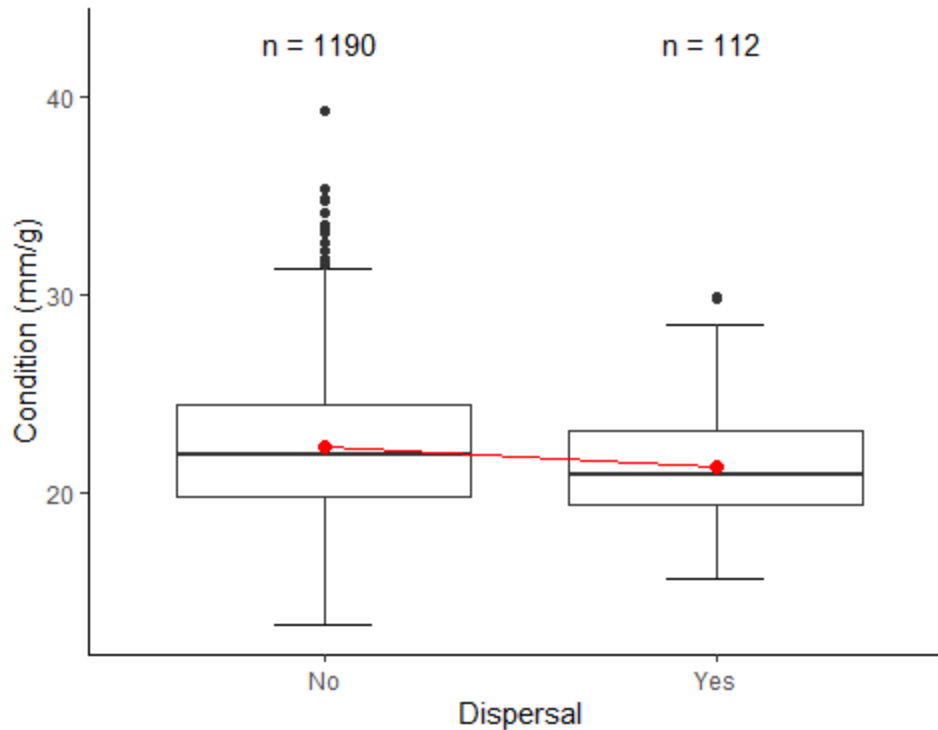
Figure 10: The condition (length/weight ratio, in mm/g) of sticklebacks that dispersed compared to those that did not. The red dots are the means. The error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile.

In the pond set-up

Size had no significant effect on neither choice of dispersal, nor number of ponds visited among fish that chose to disperse (Table 5, Fig. 11).

The probability of fish crossing was not influenced by their timing of arrival. In contrast, the number of ponds visited once dispersal had started was significantly lower for later migrants (Table 5, Fig. 12). The latency to reach the last pond, or the dispersal speed, did not covary with individual size or timing of migration (Table 6).

Table 5: Summary of the output of the models on the probability of fish crossing at least one pond, and on the amount of ponds crossed depending on condition and march date. Condition is the ratio of fish length and weight (mm/g), and March_d is March date. Estimates (β) are presented with their 95% confidence intervals and are denoted in bold if significant. Degrees of freedom (d.f) is also indicated.

	Prob fish crossed pond						Amount of ponds crossed					
Fixed effects	d.f	β	(95%CI)	d.f	β	(95%CI)	d.f	β	(95%CI)	d.f	β	(95%CI)
Intercept	256	-1.18	(-2.57, 0.37)	258	-1.02	(-2.79, 0.62)	44	**1.66**	**(1.32, 2.01)**	45	**2.05**	**(1.57, 2.52)**
Condition	256	0.02	(-0.09, 0.04)	-	-	-	44	-0.01	(-0.03, 0.01)	-	-	-
March_d	-	-	-	258	-0.01	(-0.05, 0.03)	-	-	-	45	**-0.01**	**(-0.03,-0.01)**

Table 6: Summary of the output of the models on the timing of arrival of fish depending on water temperatures ('Temp climate window') and latency to reach the last pond of fish depending on their condition (length/weight, in mm/g) and their timing of arrival (March date). Estimates (β) are presented with their 95% confidence intervals and are denoted in bold if significant.

	Timing of arrival (n=5559)		Latency (n=45)		Latency (n=46)	
Fixed effects	β	(95%CI)	β	(95%CI)	β	(95%CI)
Intercept	**44.98**	**(8.48, 81.50)**	4.867	(4.21, 5.57)	4.197	(3.092, 5.238)
Temp climate window	0.86	(-6.03, 7.75)	-	-	-	-
Condition			0.004	(-0.027, 0.032)	-	-
March date					0.017	(-0.005, 0.041)
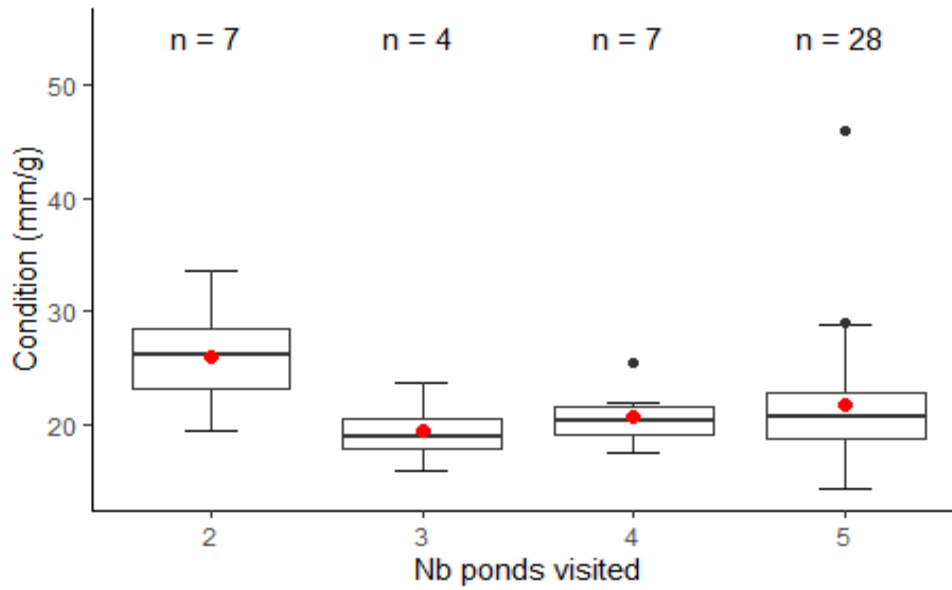
Figure 11: Relationship between stickleback condition and number of ponds visited. Condition (in mm/g) of individuals that crossed a total of 2, 3, 4 and 5 ponds. Average condition for each group is represented by the red dots, and the error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile. For each group, n is the number of sticklebacks that crossed.
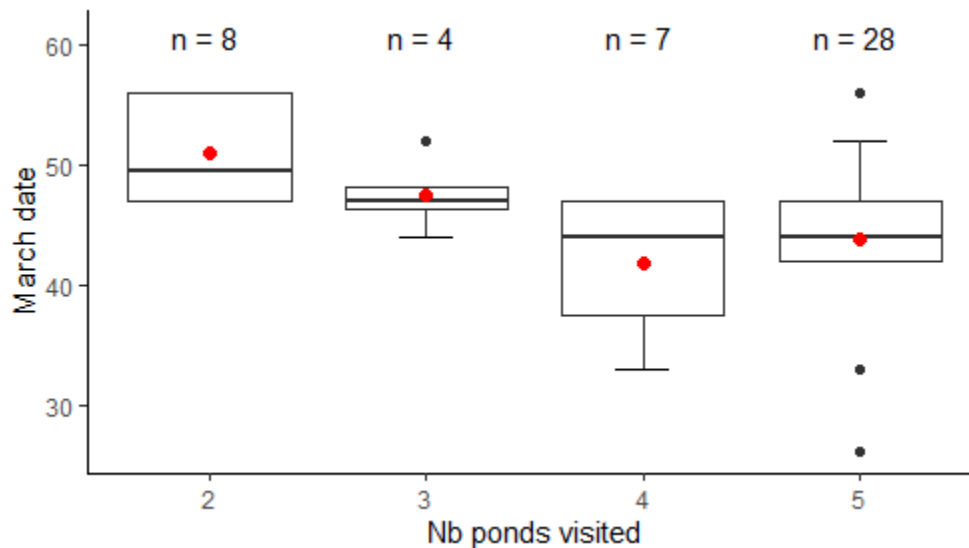

FIgure 12: Relationship between timing of arrival and number of ponds visited. Timing of individuals that crossed a total of 2, 3, 4 and 5 ponds. Average timing for each group is represented by the red dots, and the error bars represent the largest and smallest value within 1.5 times of the interquartile range above and below the 75th and 25th percentile. For each group, n is the number of sticklebacks that crossed.

Discussion

The POL syndrome hypothesis, not verified?

The main aim of his study was to assess the validity of the pace-of-life syndrome hypothesis in a population of anadromous three-spined sticklebacks. I did so through the analyses of the (co)variation of their migration timing, morphology (here, either standard length or condition depending on the data available), behaviour and dispersal upon arrival to the breeding grounds, across varying climatic conditions. Overall, I did not find that life-history strategies and behavioural traits covaried in line with the POL syndrome hypothesis. Although not sufficient to verify a POL hypothesis, some of my expectations stated in Fig. 1 were however met.

Indeed, I found a strong negative covariation between migration timing and size, as ealy fish were found to be significantly bigger than later fish. The strength of this negative relationship varied across the years. Life-history traits were therefore correlated as expected (Fig. 13). Expectations on correlations between these life-history strategies and behavioural traits were however not met. Results on boldness (assessed through exploratory tendencies) were inconsistent with our hypothesis, as smaller fish were found to be bolder than bigger ones in the wild, and no covariance was found between boldness and condition in laboratory settings. Furthermore, the link between dispersal and other traits was found either to be non-existent, or to be opposite to expectations (Fig. 13). Dispersal distance was not correlated with any other traits in the wild, but was significantly higher in fish whose migration timing was later in the pond experiments, and speed of dispersal (which aimed to measure degree of exploration) was not dependent on any of the traits measured.
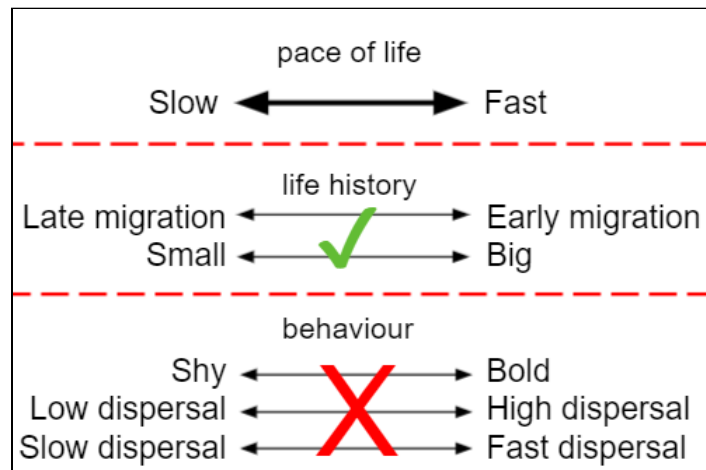
Figure 13: Schematic of the potential integration of different traits along a pace-of-life continuum, updated to reflect my results. Double arrows illustrate presumed continuous variation in life-history strategies among individuals in a population, and its assumed relationship with personality and physiological traits. The green tick shows correlation between life-history strategies, and the red cross represents the absence of link between life-history strategies and behavioural traits (based on schematic by Réale et al., (2010) and adapted to the traits of this study).

My results therefore corroborate with the conclusions put forward by Royauté *et al*., 2018 in their meta-analysis, as they established that, according to current research, behaviour is not integrated with life-history in a manner that is consistent with the POL syndrome hypothesis. Furthermore, and likewise to my study, they found that some combinations of traits were more likely to be associated in predicted directions. Their findings, together with mine, therefore support the idea that trait integration does occur but not in a consistent and predicted way, as it was observed before in other studies (Biro & Stamps, 2008; Réale *et al.,* 2010; Careau and Garland, 2012; Monceau *et al.*, 2017). This discrepancy and lack of detectable support of the POL hypothesis may be attributable to several explanations.

The POL syndrome hypothesis, a more complex story

First, the factors influencing how traits are integrated along a POL continuum are likely to be more numerous than currently considered. It is probable that behavioural traits and life histories are in fact linked, but in alternative, more complex routes than those stated and tested in this study. In fact, the current research supports the idea that substantial links between life history strategies and behavioural traits do exist, but also introduces fundamental uncertainties about the direction and mechanistic basis of these linkages (Réale *et al.,* 2010). This is especially the case seeing as the evolution of the direction of a correlation between traits is likely to vary across and within populations, depending on the ecological conditions present (Réale et al., 2010). For instance, aggressiveness and boldness was shown to be positively correlated with dispersal when the fitness of individuals relied strongly on their ability to secure a territory and survive in an unknown environment (Duckworth and Badyaev, 2007), but was also shown to be highest in philopatric individuals in habitats subject to overcompetition due to limited resources (Roff & Fairbairn, 2007). Furthermore, key traits such as dispersal tendencies may vary regardless of behavioural profiles or life-histories when they represent a clear survival advantage. This could be the case due to varying predation risks. Indeed, for example, shy individuals expected to disperse less than bold individuals of the same population might disperse equally in order to avoid predators (Cote et al., 2010). It is therefore apparent that ecological conditions have important consequences on the nature and severity of traits present within a population, and hence on how they are integrated along a POL continuum.

The POL hypothesis and varying ecological conditions

The association between specific traits in a POL syndrome is expected to vary depending on the type and severity of the ecological conditions faced by individuals. Many researchers in fact argue that the link between life-history strategies, behavioural traits and ecological conditions is likely to be highly complex, with factors such as costs of resource defence, population density, predation regime or human disturbance likely to interact and potentially alter these associations (Dingemanse et al., 2010 & 2007; Stamps and Groothuis, 2010).
Also, because individuals are likely to encounter different types of ecological conditions during their life, selection is expected to favor reversible plasticity for the traits involved in the POL syndrome (Sultan and Spencer 2002). These short-term, reversible plastic changes can affect or even hide the relationship observed among traits and therefore need to be accounted for in

order to effectively detect POL syndromes in a population (Dingemanse et al. 2012; Montiglio et al., 2014). The association between specific traits in a POL syndrome is therefore expected to vary depending on the type and severity of the ecological conditions faced by individuals of a population, and our ability to identify a link between behaviour, physiology and life history to assess the possibility of a POL syndrome will therefore highly depend on our understanding of the ecological conditions present (Nicolaus et al. 2012; Montiglio et al. 2018).

In order to account for some of the effects of varying ecological conditions, my study assessed how the direction and strength of covariation between life-history strategies and behavioural syndromes varied between the years, and with climatic conditions (here, water temperature). I expected to find some variation in trait correlation between the years, with stronger correlations in warmer years, leading to a tighter POL syndrome.

Stickleback count, migration timing and size were all shown to vary across the years, often significantly so. I therefore investigated whether some of this variation could be explained by changes in temperatures, and found that this depended on the trait (co)variation considered: migration timing was apparently unaffected by water temperatures, fish size was negatively correlated with water temperatures, and stickleback count was overall significantly lower when water temperatures approached the extremes (cold or hot). Also, migration timing was shown to covary with size, overall negatively, but differently so throughout the years.

The fact that significantly fewer fish were counted in extreme climatic conditions, together with the findings that covariation between migration timing and fish size fluctuated significantly over the years, suggests that selection pressures related to the environmental conditions indeed influenced the observed integration of traits along the POL continuum, but perhaps in ways that were not clearly reflected on the specific traits I assessed here. Namely, as water temperatures were negatively correlated with fish size, and migration timing was itself negatively correlated with fish size, it is possible that climatic conditions did impact migration timing but through more complex interaction not measured here, like food resources and/or predation risk. For instance, if food resources were limited prior to fish migration, due in part to extreme temperatures, it is possible that only a subset of the sticklebacks with specific behavioural traits managed to secure enough resources and grow fast enough in time for migration. This would lead to a 'pre-selection' of fish prior to their migration, therefore reducing the amount of trait variation within a population, and hence the potential for a POL syndrome. Previous studies have in fact shown that richer environmental conditions should favour individuals with suites of traits representative of the faster phenotypes, and oppositely poor conditions should favor slower phenotypes (Boon *et al.,* 2007; Stamps, 2007; Biro & Stamps, 2008). If this is the case, and due to this possible 'pre-selection, then in years of 'extreme' temperatures, detecting a POL syndrome would be harder as individuals present would already fall either on the high or low end of the continuum.

Consequences of my study and the POL syndrome

A better grasp of the intricacies of the POL syndrome can have important implications for the evolutionary and ecological study of populations, as it can affect our understanding of the links between behavioural traits and other important ecological, demographic and genetic factors (Réale *et al.,* 2010). It can for example help assess the direction and strength of selection acting

on traits under different environments, help predict the distribution of traits in a population depending on different ecological conditions, and it can even reflect the severity of anthropogenic disturbance faced by individuals in human influenced contexts (Réale *et al.,* 2010).

Furthermore, as covered previously, the POL syndrome is influenced by ecological conditions, and therefore most likely by the threats imposed by human-induced rapid environmental change (Bright Ross *et al.,* 2020). Better understanding how vulnerable or resilient species and populations may be to these changes is imperative if we are to hinder their impact (Pacifici *et al.,* 2015). As their capacity to adapt is inherently multifactorial, including behavioural plasticity, changes in population dynamics or life-history strategies, a comprehensive and accurate understanding of the POL syndrome could help predict these changes through their interactions on more individual levels.

In the case of the anadromous stickleback, for example, a better grasp of the POL syndrome would considerably aid in predicting how populations will persist as climatic conditions, or here water temperatures, change. This is because their fitness, more specifically reproductive success, is highly dependent on their size, which is itself dependent on the food supply and overall ecological conditions (Wootton, 1972). Larger females indeed tend to produce more eggs per spawning, have more spawnings (Wootton, 1972) and have bigger eggs (Fletcher and Wootton, 1995). Larger males also have higher reproductive success, as they are able to secure better quality territories and are therefore preferred by females, and are also better able to defend the nest from intruders (Nagel & Schluter, 1998; Kraak *et al.,* 1999; Candolin & Voigt, 2001). Additionally, Smith (1970) showed that food levels affect the ability of male stickleback to take up territories and build nests. Ecological conditions, here through food supply, therefore have an effect on population persistence via its effects on both the female and male roles in reproduction.

Limitations of the study

It is possible that certain sampling biases arose from the methods of collection of test subjects, leading to an over or under representation of individuals with particular attributes within the sample (Kressler et al., 2021). For example, current PIT tagging methods only allow for a subset of individuals to be selected, and behavioural assessments were therefore only possible on bigger individuals (>50mm). Also, the number of sampling sessions was limited and individuals that migrated earlier or later in the season might have been missed, resulting in a falsely narrow migratory window. Then, although cross nets were checked during the night, most of the fishing efforts were only carried out during the day, potentially excluding more migrants. Finally, the data gathered was somewhat fragmented as not all traits were measured every year, sometimes making yearly comparisons impossible. This was for instance the case with fish size, as small fish were only measured in 2014 and 2021. Because of these various biases in the fish collected, my assessment of the POL syndrome might have also been somewhat biased. Indeed, without a full representation of the range of traits and life-histories present among individuals of a population, only a narrow, less representative assessment of their interaction was possible. For example, I found that boldness was higher in smaller fish, but this was only

tested on already relatively large individuals, as assessing the distribution of traits among the population's smaller fish was not feasible. My conclusions might have however differed if my analyses included the traits of smaller fish. Evaluating the validity of the POL syndrome hypothesis would hence be more effective when considering the entire range of individuals in a population.

Conclusion

In conclusion, although this study found significant evidence of covariation between certain behavioural traits and life-history strategies, it also demonstrated that if the POL syndrome does occur, it is likely to be much more complex than assumed in the literature. If we are to fully validate or reject the POL hypothesis, we will need to study the relationship between these life-histories, physiologies and behaviours over various gradients of ecological conditions, including different climatic conditions, resource abundances and/or distributions, population densities and predation risks. To do so, long term studies over several generations and studies in which ecological conditions are experimentally manipulated will be needed. Although such studies might be challenging, they may be increasingly conceivable as data on behavioural traits in long-term research accumulate. A deeper evaluation of the POL syndrome could be highly beneficial to future studies, as it could help better understand the complex factors behind the selection of traits in a population, as well as their interaction with ecological conditions which are likely to be increasingly variable as anthropogenic activity continues. The POL syndrome could therefore also help predict how populations are likely to be affected by the changing climate, and in that way may help in hindering its consequences through future conservation efforts.

Acknowledgements

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Appendix

Appendix A

Schematic of the potential integration of different traits along a pace-of-life continuum. Double arrows illustrate pre- sumed continuous variation in life-history strategies among individuals in a population, and its assumed relationship with personality and physiological traits. (Réale et al., 2010)
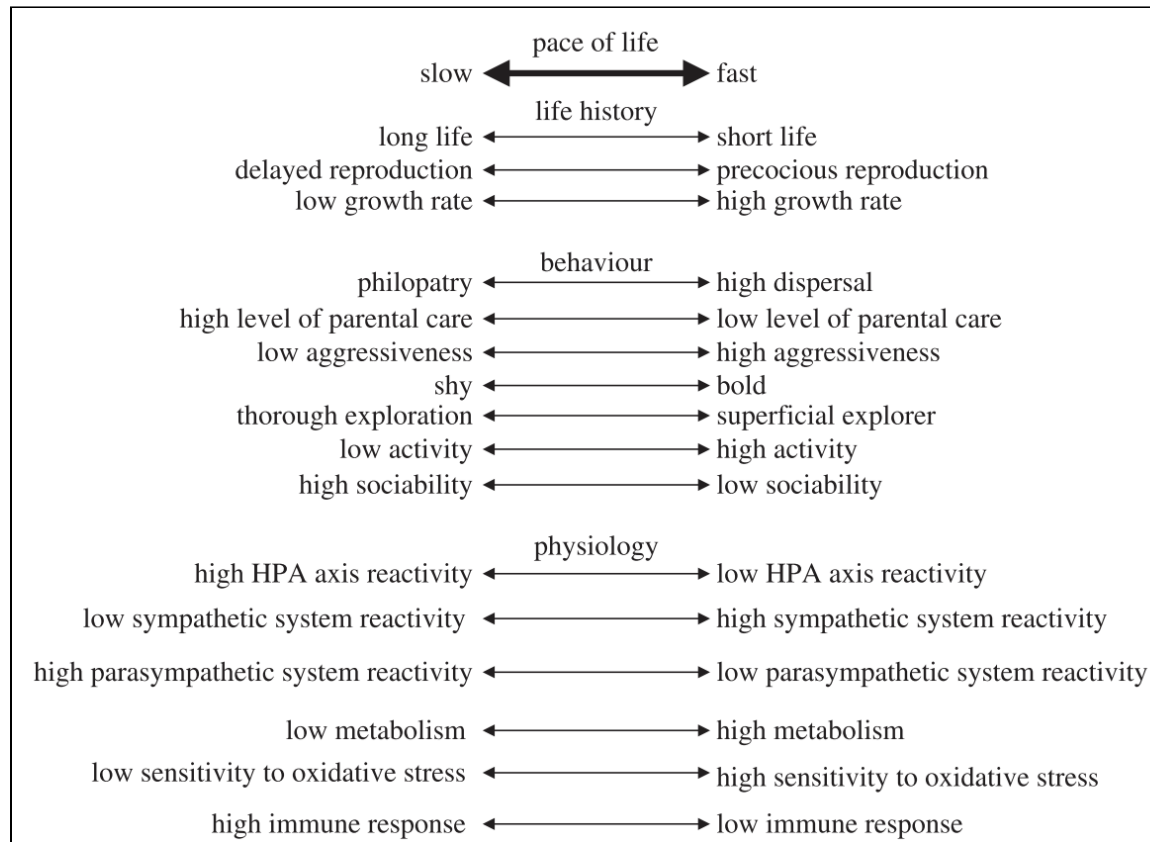
Appendix B

Map of the study area, with all five detection locations.
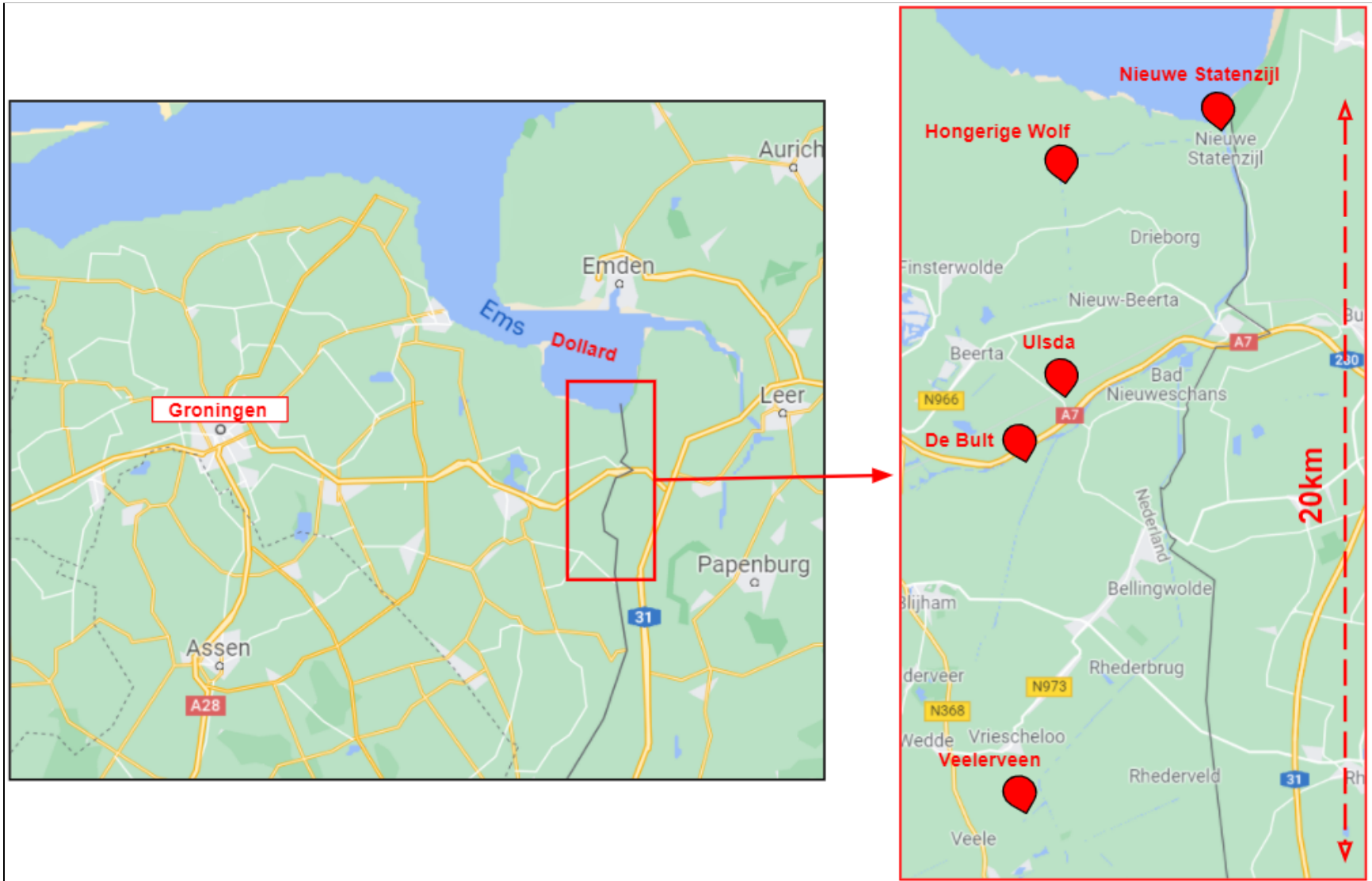
Appendix C

Poster describing the research setup in mesocosms at the university of Groningen (Mavromatika, 2021; Available at: http://mavromatika.com/en/projects/Epinoches)

Appendix D

Laboratory set-up showing the interconnected ponds. The set-up consists of two sets of 5 ponds, all connected to the same water pump. A total of 16 antennas were used, connected to two PIT readers.
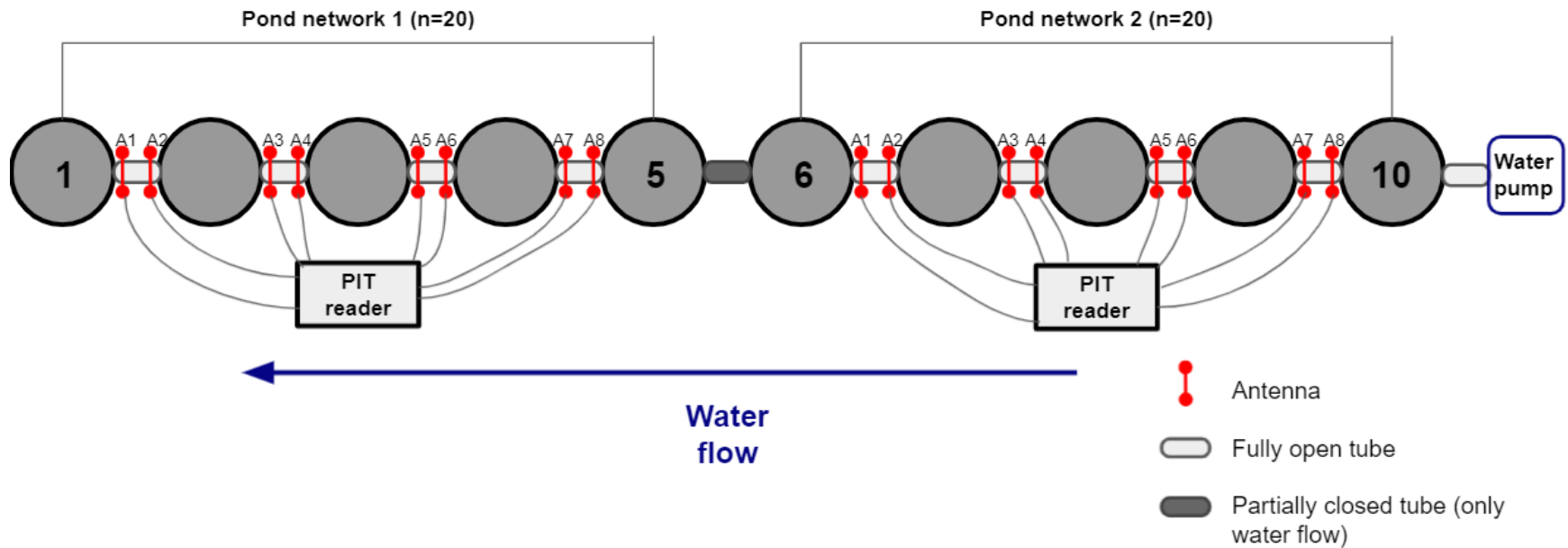
Appendix E

Output from the climate window analysis carried out on the count of sticklebacks, with a range of 4 months, and start date set at 07/03/2014 (p=0.041). Detailed explanation:https://cran.r-project.org/web/packages/climwin/vignettes/climwin.html