# Water Turbidity Affects the Development of Sexual Morphology in the Palmate Newt

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[1]In memory of Aurélien Aumjaud.

## Abstract

In theory, animal signals are designed to optimize transmission across a specific habitat. However, sexual signals characteristics often reflect habitat quality, a feature that does not necessarily match habitat structure. Besides, many species exploit a particular habitat for breeding so that the growth of sexual signals can depend on the additive effects of breeding and non-breeding habitats. We combined field and experimental data to investigate the relative effect of terrestrial and aquatic habitat on the development of sexual ornaments in the palmate newt, *Triturus helveticus*. This species exploits a large ecological range of habitats. Like many amphibians, it spends the breeding season in water and the rest of year on land. We tested the influence of terrestrial (forest cover) and aquatic habitat variables (turbidity, organic matter, pH, nitrate and chloride) on male sexual morphology. Neither terrestrial nor aquatic habitat variables accounted for body size variation. In contrast, the size of male sexual traits decreased with water turbidity, suggesting that the expression of visual signals matched the local conditions of signal transmission. We provide experimental evidence that this pattern is not caused by reduced foraging efficiency in turbid water. We propose alternative mechanisms to account for the relationship between turbidity and visual sexual signals, and discuss the consequences of small scale environmental variation on mate choice.

## Introduction

Habitat is a potent ecological factor that imposes gradual morphological changes up to the formation of distinct ecotypes (Wilding et al. 2001; Hoekstra & Nachman 2003; Yeh 2004). Animal signals are particularly sensitive to environmental variation because they are designed to optimize transmission efficiency and limit degradation across a given habitat (Marten & Marler 1977; Bradbury & Verhencamp 1998). Comparative studies revealed adaptive divergence between related taxa exploiting different habitats (Marchetti 1993; Losos & Chu 1998). At the infra-specific level, signals characteristics can vary across ecological gradients. For instance, bird species can modify their song in noisier (Slabbekoorn & Smith 2002; Slabbekoorn & Peet 2003), more closed habitats (Handford & Lougheed 1991), or more complex communities (Naugler & Ratcliffe 1994). In fishes, sexual colouration is influenced by the presence of predators (Endler 1995) or the dominant light wavelength (Fuller 2002). More generally, the size, shape or colour of a visual display primarily depend on the physical or chemical properties of habitats (Endler 1995; Endler & Théry 1996; Bradbury & Verhencamp 1998).

Yet, the influence of habitat on morphological signals can be complex. For species that exploit

different habitats during their life cycle, non-breeding and breeding habitats can drastically differ from each other but signals characteristics are expected to match the transmission properties of the breeding habitat only. Because morphological signals often start to develop before breeding (Andersson 1994), their final characteristics can be determined by the acquisition of resource in either habitats (Grether et al. 1999).

In amphibians, many species enter water for breeding and stay on land outside this period (Duellman & Trueb 1994) so that both aquatic and terrestrial habitats can influence the development of morphological traits. Growth is usually more important during non-breeding periods and stops when conditions become unfavourable (Halliday & Verrell 1986; Jakob et al. 2003). Thus, terrestrial habitat is likely to have a larger effect on overall body size than aquatic habitats. Food resource level and quality on land is likely to affect the expression of sexual traits to some extent. However, aquatic habitat is expected to influence the development of sexual characters grown during the breeding season too. Food resource availability can limit the development of sexual traits (Baker 1992) but water itself can impose severe constraints on morphology or behaviour as exemplified by the literature on endocrine disruption (Gardiner & Hoppe 1999; Noriega & Hayes 2000; Park et al. 2001; Clotfelter et al. 2004; Guillette & Edwards 2005). Turbidity is a biologically relevant factor that can disrupt sexual behaviours based on visual signals (Seehausen et al. 1997; Moyaho et al. 2004). It can also alter foraging behaviour (Utne 1997; Stuart-Smith et al. 2004; Meager et al. 2005), and potentially impede the expression of condition dependent traits.

In the genus *Triturus*, adults spend most of summer, fall and early winter on land. Then, they gather in ponds or lentic habitats where they breed, feed and stay over for several weeks to several months (Griffiths 1996; Nöllert & Nöllert 2003). Males develop dramatic sexual dimorphic traits during this aquatic phase (Halliday 1975; Griffiths 1996). Besides, there is experimental evidence that a sexual trait like the dorsal crest depends on food resource level during the breeding season (Baker 1992). Likewise, the palmate newt *Triturus helveticus* exhibit a strong sexual dimorphism. Males develop a suite of traits among which a long caudal filament, a caudal and a dorsal crest, that are suspected to be involved in courtship (Halliday 1975). For instance, female preference for males with high crest has been shown in *T. cristatus* (Green 1991) and the closely related species *T. vulgaris* (Hedlund 1990). Because of its

large ecological amplitude (Morand & Joly 1995; this study), the palmate newt provides a good model to test the effect of habitat on morphology.

We investigated the influence of terrestrial and aquatic habitat characteristics on the variation of sexual and non-sexual morphological traits. Mostly, newts on land exploit wooded habitats where they find food and shelter (Griffiths 1996). We therefore predict body and sexual trait size to increase with forest cover in pond vicinity. The effect of aquatic habitats on sexual traits is more complex. Those traits are expected to increase in size with habitat productivity. However, human activities can cause disturbances by introducing pollutants or endocrine disruptors in ponds. Thus, we predict a negative relationship between ornament size and concentration of ions used in agriculture. Finally, light transmission characteristics in water is likely to alter foraging and visual signalling efficiency. Turbidity is due to the presence of suspended particles that block light transmission. Although naturally occurring, this process can be generated by human activities (in ponds for watering cattle for instance). Turbidity causes a reduction of contrast between background and body shape or colour patterns. It also limit prey detectability for visual predators. Consequences for organisms lead to opposite predictions. On the one hand, larger sexual traits are expected in turbid waters to compensate the loss of signalling efficiency. On the other hand, lower foraging efficiency in turbid water may limit trait expression. To test these predictions we analysed the relationship between turbidity and morphological characteristics. In addition, we experimentally measured the effect of turbidity on foraging efficiency. We measured prey capture rate of two prey types, cladoceran adult (*Daphnia pulex*) and chironomid larvae, in adults newts exposed to turbid and non-turbid water conditions.

## Materials and Methods

### Populations

We sampled 11 breeding ponds in Maine-et-loire near Angers (47°28′N latitude, 0°33′W longitude) in western France (Fig. 1) and collected 205 male palmate newts between 08/03/05 and 02/05/05. Sexual characters are grown when adults enter water and regress rapidly when they leave the aquatic habitat (Griffiths & Mylotte 1988). The study was carried out when the development of sexual traits was maximal. Six sites were located in the floodplains of the Loire and Loir rivers close to the
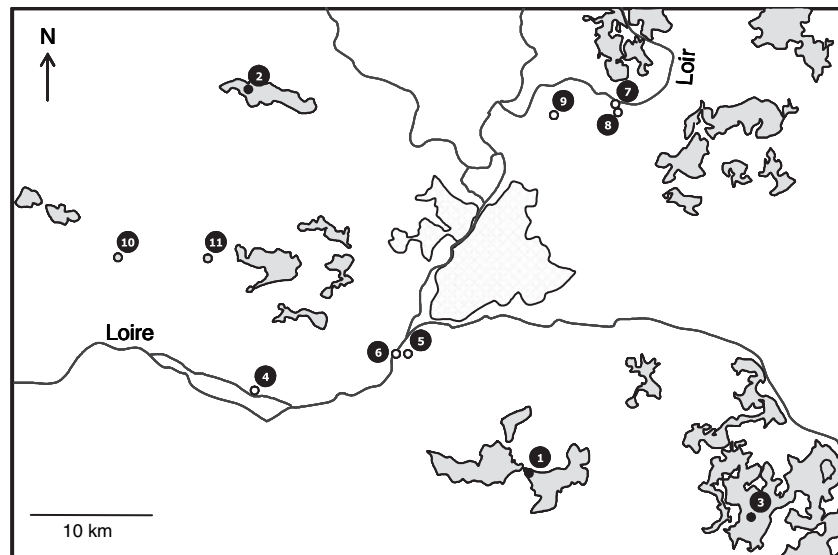
**Fig. 1:** Location of the 11 breeding ponds sampled for this study. Figures refer to site numbers (see Table 1). Black circles: forest ponds. White circles: floodplain pond. Grey circles: agricultural plateau ponds. Shaded areas show the main forest patches. The large patterned area is the urbanized area covered by the town of Angers

**Table 1:** Habitat characteristics of the 11 breeding ponds sampled in this study

| Site ID | Habitat | n | Forest cover (ha) | Turbidity (NTU) | Organic matter (UV 254 nm) | Conductivity ($\mu$S/cm) | pH | Cl− (mg/l) | NO3- (mg/l) | SO4- (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Forest | 34 | 69.72 | 6.5 | 0.532 | 196 | 7.22 | 53.83 | 1.067 | 28.796 |
| 2 | Forest | 20 | 73.18 | 29 | 0.762 | 94 | 7.16 | 15.72 | 0.676 | 6.491 |
| 3 | Forest | 32 | 72.55 | 3.2 | 0.905 | 45 | 6.74 | 4.44 | nd | nd |
| 4 | Floodplain | 31 | 5.60 | 7.6 | 0.53 | 570 | 7.65 | 5.86 | 0.152 | 97.251 |
| 5 | Floodplain | 22 | 6.10 | 8.1 | 0.343 | 386 | 7.7 | 147.21 | 8.718 | 64.044 |
| 6 | Floodplain | 23 | 3.64 | 1.5 | 0.276 | 622 | 8.37 | 149.22 | 22.376 | 67.889 |
| 7 | Floodplain | 5 | 9.28 | 1.27 | 0.618 | 414 | 9.26 | 273.79 | 0.504 | 294.653 |
| 8 | Floodplain | 5 | 11.00 | 1.35 | 1.073 | 1075 | 7.98 | 63.977 | 1.043 | 616.567 |
| 9 | Floodplain | 21 | 14.89 | 2.5 | 0.622 | 360 | 9.79 | 60.39 | nd | 89.101 |
| 10 | Agricultural | 5 | 1.37 | 22 | 0.481 | 453 | 7.82 | 45.93 | nd | nd |
| 11 | Agricultural | 7 | 0.03 | 9 | 0.632 | 279 | 7.31 | 58.13 | nd | 46.774 |

n, number of males collected in each pond; ID, label assigned to each pond; nd, not detected.

watercourse; three were inside large forest patches. The two remaining sites were on the plateau, and were mainly surrounded by meadows and cultures. Sample sizes for all sites are given in Table 1. Distance between sites ranged between 0.2 and 50 km. All sites were approximately included in a 50 × 30 km area. Individuals were caught using dip nets and brought to the laboratory. All newts were kept in captivity between 2 d and 6 wk and released on their breeding pond. Permits were delivered by Préfecture du Maine-et-Loire.

### Terrestrial and Aquatic Habitat Measurement

We quantified terrestrial habitat characteristics by measuring the surface of forested habitat within a 500 m-radius around each pond using aerial photographs (Orthophotos IGN 1/25000) and the software MAPINFO 6.0. We selected this scale because *Triturus* newts usually remain a few hundred meters of the breeding pond during the terrestrial phase (Jehle 2000).

Water samples of 1.5 l were taken from each pond on 24/05/05. Because of time and cost constraints, one sample per pond could be analysed. Suspended solids (SS) were analysed according to French standard procedures (AFNOR NF T 90-105) by filtration under specified conditions (glass fibber filter: porosity 0.7 $\mu$m, drying at 105°C and weighting). Water turbidity (TUR) measures the clarity of water and was recorded using a portable turbidimeter (HACH). The light transmittance of samples was given in Nephelometric Turbidity Units (NTU). We estimated the amount of organic matter (OM) by measuring UV absorbance at 254 nm with a UV spectrophotometer (UNICAM). OM is representative of aromatic structures present in organic substances and can be

considered as an indirect measurement of habitat productivity. Conductivity and pH were recorded using a portable conductivity meter and pH meter (CONSORT model 831, Turnhout, Belgium) equipped respectively with a conductivity cell and glass and calomel electrodes. Concentrations in nitrate ($NO_3^-$), sulphate ($SO_4^{2-}$) and choride ($Cl^-$) ions were quantified by ionic chromatography composed by a HPLC pump (water 501) and a conductimetric detector (Waters 431). A Grace Vyade column (Type 302IC4.6, $0.46 \times 25$ cm) was used with a MeOH/$H_2O$ mixture (pH 6.8) as an eluent. For statistical convenience, concentrations under the threshold detections have been considered as null. Nitrate, sulphate and chloride can naturally occur in ponds often at low concentration. We use them here as proxies of the influence of agricultural activity on water characteristics.

## Morphological Measurements

We recorded seven morphological measurements. Body mass (BM), snout-vent length (SVL), head width (HW) and tail length (TL) will be referred hereafter as non-sexual characters. Although some of these variable vary seasonally (Griffiths & Mylotte 1988) and are sexually dimorphic, they can be considered as general morphological traits observed in both sexes and expressed all year round. In contrast, maximum tail height (MTH), filament length (FIL), and hind feet web (HFW) were considered as sexual characters because they are only expressed during the breeding season and are known, or suspected, to be involved in mate choice (Halliday 1975, J Secondi unpubl. data). Beside, MTH and HFW are present in males only. FIL is also expressed in females but its size is reduced.

Snout-vent length was measured as the distance from the tip of the snout to the anterior end of the cloaca. HW was the maximal head width posterior to eye. TL was the distance from the posterior end of the cloaca to the posterior end of the tail discarding the filament. MTH was the maximal distance between the ventral and dorsal sides of the caudal crest. FIL was the distance between the posterior end of the tail and the posterior end of the filament. HFW was the surface area of the extended hind feet web. All distances were measured in two successive series using a digital calliper to the closest 0.01 mm. BM was measured using a precision scale to the closest 0.01 g. Pictures of the hind feet web extended across a measuring paper (grid 1 mm$^2$) were taken using a 5 million pixel digital camera (Powershot A95, Canon Inc., Japan). HFW was then measured using

**Table 2:** Computation of intra-class correlation coefficients (r). Component loadings and percentage of variance explained for the first two axes of a principal component analysis on seven morphological variables

| Variable | Repeatability | | | | PCA | |
|---|---|---|---|---|---|---|
| | MSw | MSb | $n_0$ | r | PC1 | PC2 |
| BM[a] | – | – | – | – | 0.937 | 0.154 |
| SVL[a] | 0.040 | 4.400 | 30 | 0.784 | 0.904 | 0.265 |
| HW | 0.008 | 0.147 | 30 | 0.367 | 0.842 | 0.236 |
| TL | 0.020 | 8.110 | 30 | 0.931 | 0.846 | 0.311 |
| MTH[a] | 0.100 | 0.512 | 30 | 0.626 | 0.341 | −0.731 |
| FIL[a] | 0.009 | 2.738 | 30 | 0.91 | 0.305 | −0.777 |
| HFW[a] | 0.040 | 28.740 | 30 | 0.96 | 0.588 | −0.611 |
| % variance explained | | | | | 52.51 | 25.13 |

PC1 is a measure of overall body size whereas PC2 represents the size of sexual characters (MTH, FIL, HFW).
BM, Body mass; SVL, snout-vent length; HW, head width; TL, tail length; MTH, maximum tail height; FIL, filament length; HFW, hind feet web.
[a]Indicates that residuals of regression of character size on date have been used in the PCA (see Material and Methods). Only raw data have been used for repeatability measurements.

the software IMAQ Vision Builder (NATIONAL INSTRUMENT, Austin, TX, USA). Repeatabilities were calculated using intra-class correlation coefficient as described in Lessells & Boag (1987) for all variables but BM. For this variable, the spring precision was high ($\pm0.01$ g). After weighing few individuals twice we decided to take only one measurement. Because measuring HFW is a time-consuming procedure, we took double measurements on a random sample of 30 males. We computed repeatability for all character using this sample. All variables were moderately to highly repeatable (Table 2). For statistical analyses, we used the mean value of the two measurements. Morphological characteristics were measured within 48 h after capture. Individuals were anaesthetized prior to manipulation by immersion in Tricaine methane sulphonate (0.2 g/l), MS222, during 5–15 min. Every male recovered within a few hours after measurements.

## Foraging Experiments

Thirty males from population five were caught with dip nets on 25/04/06 to test the effect of water turbidity on foraging efficiency. We tested two prey types, cladoceran adults (*Daphnia pulex*) and chironomid larvae, that differ by their mobility and their position in the water column. *Daphnia* continuously swim in the water column while chironomid remain on the bottom. In addition, chironomids display a red body colouration that cladocerans did not exhibit in the population we used. Cladocerans often constitute

dominant prey items of small *Triturus* species (Joly & Giacoma 1992). However, both prey types are regularly consumed by the palmate newt, a species that seems to show low food selection (Griffiths 1986).

Turbidity level were set to 0 and 25 NTU which is close to the upper value observed on the 11 ponds analysed here. To set the turbidity level, we added 0.2 g/l of bentonite, a biologically neutral clay, to dechlorinated tap water.

Foraging tests were carried out in aquaria (45 cm × 20 cm × 30 cm) containing 2 l of water. Before each test, individuals were left unfed for 20 h. We provided males with ten *Daphnia* in the first experiment and six chironomid larvae in the second one. Size difference between cladocerans and chironomids accounted for the difference in the number of items provided. Tests lasted 4 h between 15:00 and 19:00. We used a repeated measurement design in which male newts were presented the two treatments (turbid or clear water) on two different sessions. *Daphnia* experiments were carried out on the 28/04 and 29/04, chironomid experiments on the 01/05 and 03/05. In both experiments, treatment order (clear/turbid) was randomized between males. In order to maintain turbidity during the whole experiment, water was gently mixed after 2 h. The same procedure was applied for the two treatments.

### Statistical Analyses

#### Effect of Capture Date on Morphological Characters

Some traits vary during the breeding season. In particular, sexual traits develop when individuals enter water and regress rapidly when they leave (Griffiths & Mylotte 1988). We computed linear regressions of each trait on capture date and found a significant increase in BM, SVL, MTH, FIL and HFW with date. For those, capture date might have been a confounding factor. Thus, all further analyses were carried out using the residuals of regressions for these five variables. Raw data were used when no significant linear relationship was observed.

#### Effect of Habitat on Morphology

We carried out a principal component analysis (PCA) on all morphological variables. This technique extracts components that are general and independent estimates of individual morphology, and reduces the risk of type I error by limiting the number of statistical tests to carry out. We first tested morphological variation between sites using an ANOVA on PCA variables. We then tested the effect of habitat varia-

**Table 3:** Matrix of Pearson correlation coefficients for habitat variables measured in 11 breeding ponds. Correlations have been computed on log-transformed data for variables 2, 4, 6, 7, 8

|  | (1) | (2) | (3) | (4) | (5) | (6) | (7) |
|---|---|---|---|---|---|---|---|
| % forest (1) | | | | | | | |
| Turbidity (2) | 0.241 | | | | | | |
| Organic matter (3) | 0.384 | −0.178 | | | | | |
| Conductivity (4) | **−0.833** | −0.354 | −0.253 | | | | |
| pH (5) | −0.521 | −0.547 | −0.196 | 0.529 | | | |
| Chloride (6) | −0.509 | −0.384 | −0.406 | 0.553 | 0.589 | | |
| Nitrate (7) | −0.181 | −0.224 | −0.492 | 0.150 | 0.064 | 0.535 | |
| Sulphate (8) | −0.483 | **−0.613** | 0.015 | **0.699** | 0.543 | 0.532 | 0.030 |

Bold figures indicate significant p-values ($\alpha = 0.05$).

bles (see below) on morphology, i.e. on PCA axes, using linear mixed-effects models (LME). Because newts originated from different ponds (or site) in a habitat gradient, we considered site as a random effect and fitted models by the restricted maximum log-likelihood (REML). From the two complete models, we selected minimal models using a backward selection procedure that removed the least significant factor at each step ($p > 0.05$). PC2 was square root transformed before testing to meet the criteria of normality and homoscedasticity. To ensure all values were positive prior to transformation, we added three units to individual scores. Analyses were carried out using STATISTICA 5.1 (Statsoft) and R (R Development Core Team 2004).

The number of habitat variables was large relative to the number of individuals. Because such a situation can limit analysis reliability, we reduced the set of independent variables. Variable selection was carried out using a correlation analysis on all independent variables (Table 3). Variables 2, 4, 6, 7, 8 have been log-transformed to improve linearity between variables.

To test for a significant relationship between geographical and morphological distances in the population sample, we carried out a Mantel test using POPTOOL 2.6 (Hood 2005).

#### Foraging Tests

We used Wilcoxon signed rank test to test the difference in foraging efficiency between clear and turbid water.

### Results

#### Description of Male Morphology

Principal component analysis generates synthetic and independent variables of male morphology.

The two first PCA axes (Table 2) accounted for 77.64% of variance. All loadings were positive on the first axis PC1 that represents a measure of overall body size. In contrast, loadings were positive for non-sexual characters (BM, HW, SVL, TL) and negative for sexual characters (MTH, FIL, HFW) on PC2. Moreover, the absolute values of sexual characters loadings were two to five times larger than loadings of non-sexual characters. Thus, PC2 provides a measurement of the relative size of morphological secondary sexual traits. We observed a significant variation in non-sexual and sexual male morphology between populations (ANOVA, respectively PC1 and PC2: $F_{10,194} = 10.525$, $p < 0.001$; $F_{10,194} = 22.890$, $p < 0.001$).

## Effect of Terrestrial and Aquatic Habitat on Morphology

Many variables were correlated with one or several others. In order to prevent any problem caused by collinearity, we removed one variable among those pairs whose correlation coefficient tests yielded a p-value lower than 0.05 (Table 3). We observed a strong significant negative relationship between conductivity and forest cover within a 500-m radius around the pond. Therefore, conductivity can be considered as an indicator of chemical processes associated with the accumulation and transformation of organic matter, and more largely of the surrounding terrestrial habitat. Sulphate was correlated negatively with turbidity and positively with conductivity. We discarded conductivity and sulphate concentration from the analysis and eventually retained six independent variables: forest cover, turbidity, organic matter, pH, nitrate and chloride concentration.

We used PCA variables to test the effect of habitat on morphology. We tested the effects of these six variables on PC1 and PC2 using mixed effect model (Table 4). None of these variables had a significant effect on PC1. Yet, we observed a positive trend between male size and forest cover (p = 0.08). In this analysis, population 6 (see Table 1) stands out regarding the size of individuals. A significant effect of forest cover is observed when this population is discarded from the analysis (p = 0.02, data not shown).

For PC2, the final model retained turbidity only. The five other variables were not significant and were consequently dropped from the analysis (Table 4). The relationship between turbidity and PC2 was positive (Table 4, Fig. 2) which means that the size of sexual characters decreased with increasing levels of water turbidity.

**Table 4:** Minimal models testing the effect of one aquatic and one terrestrial habitat variables on male morphology. PC1 represents body size and PC2 the size of sexual characters. Each minimal model have been selected from a complete model including six aquatic and terrestrial habitat variables using linear mixed-effects models fitted by REML with a backward selection procedure (see Material and Methods). ($n_{individual} = 205$, $n_{site} = 11$)

|  | Coefficient | SE | numDF | denDF | F | p |
|---|---|---|---|---|---|---|
| PC1 (body size) |  |  |  |  |  |  |
| Intercept | −0.305 | 0.223 | 1 | 194 | 1.874 | 0.1726 |
| Forest cover | 0.011 | 0.005 | 1 | 9 | 3.76 | 0.0844 |
| PC2 (sexual characters) |  |  |  |  |  |  |
| Intercept | 1.468 | 0.096 | 1 | 194 | 1177.058 | <0.001 |
| Log (turbidity) | 0.404 | 0.116 | 1 | 9 | 12.155 | 0.007 |

Neighbouring populations can be more similar morphologically than distant populations because of the homogenizing effect of gene flow or because they experience more similar environmental conditions. However, we observed no significant relationship between geographical and morphological distances (Mantel tests, PC1: p = 0.426, PC2: p = 0.185).

## Foraging Experiments

At the end of the *Daphnia* experiment, the proportions of remaining prey were 0.90 (±0.16 SD) in and 0.86 (±0.24 SD) respectively in turbid and clear water treatments. They were 0.79 (±0.35 SD) in turbid water and 0.74 (±0.38 SD) and clear water treatment at the end of the chironomid experiment. We detected no effect of turbidity level on foraging efficiency both in the *Daphnia* (Wilcoxon signed rank test: n = 30, z = 0.823, p = 0.410) and the chironomid experiments (Wilcoxon signed rank test: n = 30, z = 0.738, p = 0.460) (see Fig. 3).

## Discussion

### Habitat-Dependent Variations in Male Morphology

We detected no significant effect of terrestrial habitat on male morphology. Yet, we observed a trend suggesting that body size was larger in forested than in more open habitats. Earlier studies reported size differences between ponds surrounded by different habitat types (Beebee 1983) and a correlation between the species occurrence and the amount of non-cultivated land around breeding sites (Joly et al. 2001). Both studies support a general effect of terrestrial environment on newt morphology. Size variation in adults probably reflects the fact that
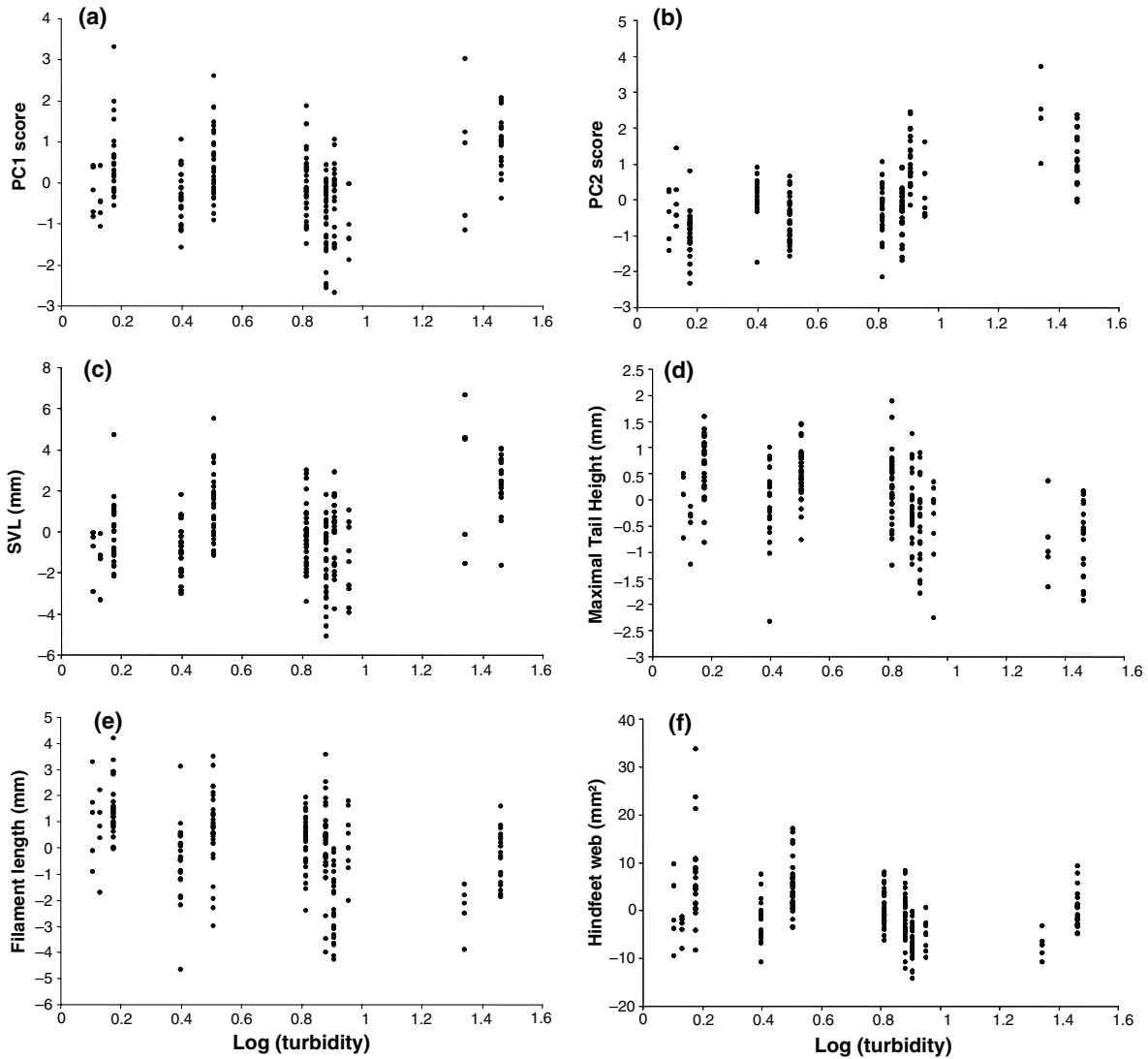
**Fig. 2:** Relationships between water turbidity and the size of non-sexual (a) and sexual morphological traits (note that untransformed PC scores are shown), (b) and in male palmate newts. Graphs c-f show variation for individual traits: snout-vent length (SVL), maximal tail height, filament length and hindfeet web surface area. Residuals of a regression on capture date has been used for all traits (see Material and methods)

differences in habitat quality affect growth or survival. We observed no significant effect of aquatic habitat on non-sexual morphology either.

In contrast, we detected a strong effect of aquatic habitat on sexual morphology as the size of sexual traits decreased with increasing water turbidity. *Triturus* newts mostly use chemical and visual communication (Malacarne & Vellano 1987; Halliday 1990; Hedlund 1990; Denoël 1999; Secondi et al. 2005) and sexual selection is believed to account for the ornaments observed in this genus (Malacarne & Cortassa 1983; Hedlund 1990; Baker 1992). The gradual variation of sexual trait size across a turbidity gradient suggests that ornament size matches the

conditions of light transmission in ponds. This result does not support the hypothesis predicting that males with larger ornaments should be observed in turbid water. Data show that males do not compensate poor conditions of light transmission by growing larger attributes. Few other cases of fine match between signal characteristics and environment across populations like the relationship between breeding colouration and predation risk in guppies *Poecilia reticulata* (Endler 1995) or the match between trill rate and habitat openness in *Zonotrichia capensis* (Handford & Lougheed 1991), have been reported. Predation has not been analysed in this study. Individuals with larger attributes could be
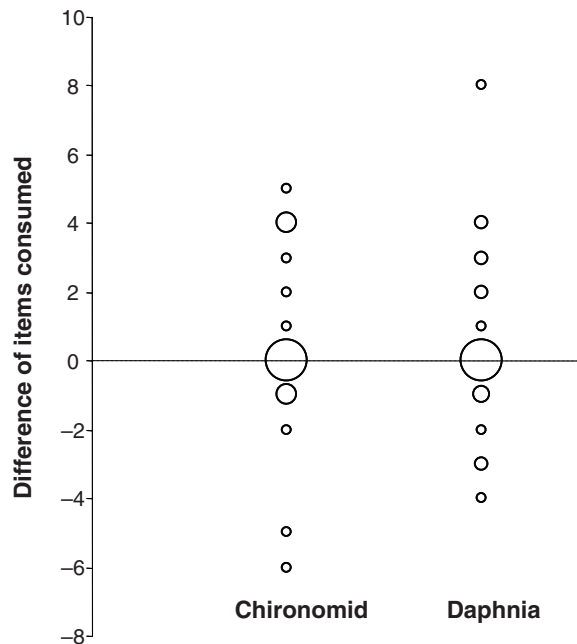
**Fig. 3:** Difference of prey items consumed between turbid and clear water treatments in the two foraging experiments. A positive value indicates that a higher number of preys has been captured in the clear water treatment. Dot area is proportional to the number of individuals that obtained that score

preferentially preyed upon by predators like fish and birds. These are common on open breeding sites, especially in floodplains, but they were not observed in forest habitats. Although predation risk is probably higher in former sites, individuals did not exhibit reduced sexual characters.

Besides, turbidity was not correlated to forest cover. More generally, no other confounding ecological factor seems to explain morphological variation of sexual traits.

### Turbidity and the Expression of Sexual Ornaments

Turbidity is expected to disturb visual communication by decreasing signalling efficiency (Seehausen et al. 1997). The mechanism underlying morphological variation is not straightforward though. Spatial and temporal changes in ecological conditions on breeding habitats can potentially affect resource acquisition and resource allocation to sexual traits. How populations cope with such environmental variability remains largely undescribed. Selection and phenotypic plasticity can account for the variation observed in our study.

Habitat-dependent selection could adjust the expression of visual signals to the local conditions of

signal transmission. However, such a mechanism implies that ponds are stable over time and that gene flow between breeding sites is low enough not to disrupt local adaptations. These assumptions are probably not to be met. Ponds and their surroundings change over time. Organic matter accumulates and the aquatic and terrestrial vegetation cover can vary dramatically within a few years. Besides, morphological variation took place on a relatively small geographical scale in a continuous mosaic of habitats (cultures, meadows, vineyards, woodlands) over a flat agricultural landscape with no other major geographical barriers than rivers. The palmate newt is common in this part of its distribution range and occurs in most of these habitats. In such a biogeographical context, it is unlikely that newt populations are isolated, or have been for long, from each other. Thus, local adaptation via selection does not convincingly explain the match between phenotype and environment.

The same reasons that tend to discard selection, i.e. the mosaic landscape structure and the temporal variation of breeding habitats, support a role for phenotypic plasticity. The plastic development of sexual ornaments could occur in various ways. First, because *Triturus* newts use vision to detect prey (Griffiths 1996), lower foraging efficiency in turbid waters could reduce the amount of available resource for visual signals. Experimental data do not support this hypothesis. Turbidity, which was set to the upper limit observed on our breeding sites, did not affect prey capture rate. Newts, like other species (Meager et al. 2005), may use other senses like electroreception (Himstedt et al. 1982; Griffiths 1996) and olfaction (Placyk & Graves 2002) to compensate bad visual conditions. Moreover, this species does not actively chase preys and capture opportunistically individuals passing at close range instead. Thus, turbidity might only marginally reduces foraging efficiency. Second, habitat quality may decrease with turbidity. This factor could reduce primary productivity and set a limit to the food resource level. Adult newts are generalist carnivorous so that food resource level may be not strongly linked to primary productivity but this hypothesis cannot be rejected. More generally, we cannot discard the possibility that turbididity is associated with anthropogenic disturbances. In agricultural landscapes the intensity or the frequency of anthropogenic disturbances, like contamination by pollutants or mechanical perturbation by cattle, is likely to alter habitat quality to various degrees. To this regard, we observed a significant correlation between turbidity and sulphate

concentration. Thus, the negative relationship between the size of sexual traits and turbidity might reflect general better ecological conditions for newts in clear water ponds which allow them to grow larger sexual ornaments. Finally, individuals might adjust the expression of sexual ornaments to the conditions encountered each breeding season. In turbid environment, males might limit their investment in inefficient visual signals and re-allocate resource to other activities or functions like searching time for partners or sex pheromone production. The major effect of an abiotic factor, like turbidity, on the expression of sexual traits is an hypothesis that requires further testing but it opens an interesting issue about the proximate mechanisms regulating investment in sexual signals.

The effect of aquatic habitat on sexual morphology in the palmate newt has broader theoretical implications. Annual or spatial changes in the expression of male sexual trait is likely to select for plasticity in female preferences as the relative size of sexual traits would vary over short periods of time or small spatial scales. This raises questions about the process of sexual selection in heterogeneous environments like mosaic landscapes. How female preferences are affected by habitat-dependent sexual signalling and what are the consequences on the mate selection process remain to be investigated.

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