International Baccalaureate

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Title of the extended essay: The Effect of Light Intensity, Temperature and pH on the Level of Physical Activity of Dragonfly Nymphs (Anisoptera)

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I.B. Extended Essay

Submitted 6 December 2007

Biology HL

The Effect of Light Intensity, Temperature and pH on the Level of Physical Activity of Dragonfly Nymphs (*Anisoptera*)

Word Count: 2,990

Table of contents

1. Abstract

Through a series of ex-situ experiments to explore the effects of human activity on other living organisms, this study aimed to answer the question of how light intensity, temperature and pH affect the level of physical activity of dragonfly nymphs.

The experiments recorded the mean duration of movement, the mean number of directional changes and the mean number of episodes (starts) of movement of 10 nymphs in each trial. The nymphs were exposed to light intensities ranging from dim to bright, temperatures from 10.2°C to 31.3°C and pHs from 4.9 to 8.9. Z-tests were used to determine the significance of the differences between the different means within the trials.

Positive correlations were found between duration of movement and water temperature, number of directional changes and light intensity, and the number of movement episodes and water temperature. These results indicated that high temperature promoted hunting behaviour in the nymphs and that high light intensity provoked a flight reaction. The majority of results, however, did not show a significant pattern and therefore could not be used to draw any solid conclusions.

(179 words)

2. Introduction

I am concerned about global warming and pollution and their impacts on living organisms. However, these issues are too broad to be explored in sufficient detail within the extended essay. Consequently, the topic was narrowed down to an investigation consisting of a series of experiments and analysis of a single species' behavioural response to variations in three fundamental environmental factors: light intensity, temperature and pH. I decided to use dragonfly nymphs (Order: *Odonata*, Suborder: *Anisoptera* [Encyclopædia Britannica, 2007]), because movement is easily observable in them as they are fairly large and commonly found in ponds.

My research question is, therefore, "**How do light intensity, temperature and pH affect the level of physical activity of dragonfly nymphs?**"

These three characteristics of aquatic environments are affected by global warming and pollution. I chose the nymphs' level of physical activity as the dependent variable because it is easily observable with the naked eye. Measuring direction and speed of movement were not feasible using the available equipment.

In this investigation consisting of a series of experiments, dragonfly nymph movement was the dependent variable. It was measured in three ways:

1. Overall time spent travelling as a percentage of the duration of the observation,
2. Number of changes in direction while travelling,
3. Number of episodes (starts) of movement

Research on this specific topic was difficult, as dragonfly nymphs seem seldom the subject of professional studies in this field. Nevertheless it was possible to find related investigations involving the effects of temperature and pH on other aquatic organisms, as well as on specific aspects of dragonfly nymph behaviour. I had no success in acquiring research articles concerning the effects of light on aquatic heterotrophic organisms.

One particular study (Gorham and Vodopich, 1992) describes the relationship between water pH and the rate of predation by damselfly nymphs. In the study, damselfly nymphs tolerated pHs as low as 4.5

without significant effects. However, at pH 3.5, their predation rate decreased significantly and their fitness was greatly reduced (respiration rate increased).
Another investigation (Pandian, Mathavan, and Jeyagopal, 1979) studied the effects of water temperature on the rate of mosquito predation by dragonfly nymphs. In this investigation, rising water temperatures saw an increase of predation.

A different study (Calaban and Makarewicz) investigated movement of daphnia within specific temperature gradients in the water. It found that *Daphnia magna*'s vertical migration "may be reduced by an avoidance reaction to cold water temperatures."
This might indicate that since *Daphnia*, prey of dragonfly nymphs, are most likely to be found in warm water, dragonfly nymphs move more when in warm water to hunt. This is supported by the apparent increase in predation in Pandian, Mathavan, and Jeyagopal's study (1979) involving mosquitoes.

3. Hypothesis

Dragonfly larvae likely move for longer periods (duration) with more directional changes (turns) and fewer stops (episodes) under stressful conditions in order to escape threats to their survival. Threats to survival are assumed to include excessive heat, excessive acidity or alkalinity, and excessive light. Extremes in temperature, acidity or alkalinity are presumed to provoke a flight response due to their direct threat to the dragonfly nymph's health whereas excessive light could be perceived as a dangerous environmental condition in which the nymphs are visible and therefore particularly vulnerable to predation.

4. Variables

Independent

- Light intensity
- Water temperature
- Water pH

Dependent

- Net duration of movement of dragonfly nymphs
- Number of directional changes in movement of dragonfly nymphs
- Number of episodes of movement of dragonfly nymphs (discreet periods of movement interrupted by periods of idleness)

Controlled

For all experiments:

- Water depth in testing aquarium
- Water source (Alte Donau)
- Dimensions of testing aquarium

Additional controlled variables for light experiment:

- Water temperature (= 21.5°C)
- Water pH (= 6.9)

Additional controlled variables for temperature experiment:

- Light intensity (dim)
- Water pH (= 6.9)

Additional controlled variables for pH experiment:

- Water temperature (= 21.5°C)
- Light intensity (dim)

5. Apparatus

For all experiments:

- Ice chest (as a reservoir of pond water from the Alte Donau)
- 1 plastic testing aquarium (31×21×20 cm)
- 2 plastic home aquaria ([31×21×20 cm] and [25×16×15 cm])
- 1 large plastic Tupperware
- Digital thermometer
- Laptop PC
- Stopwatch program
- 2 litre glass measuring cup
- 10 dragonfly nymphs
- Starting ring (cut section of a plastic bottle)

Additional apparatus for light investigation:

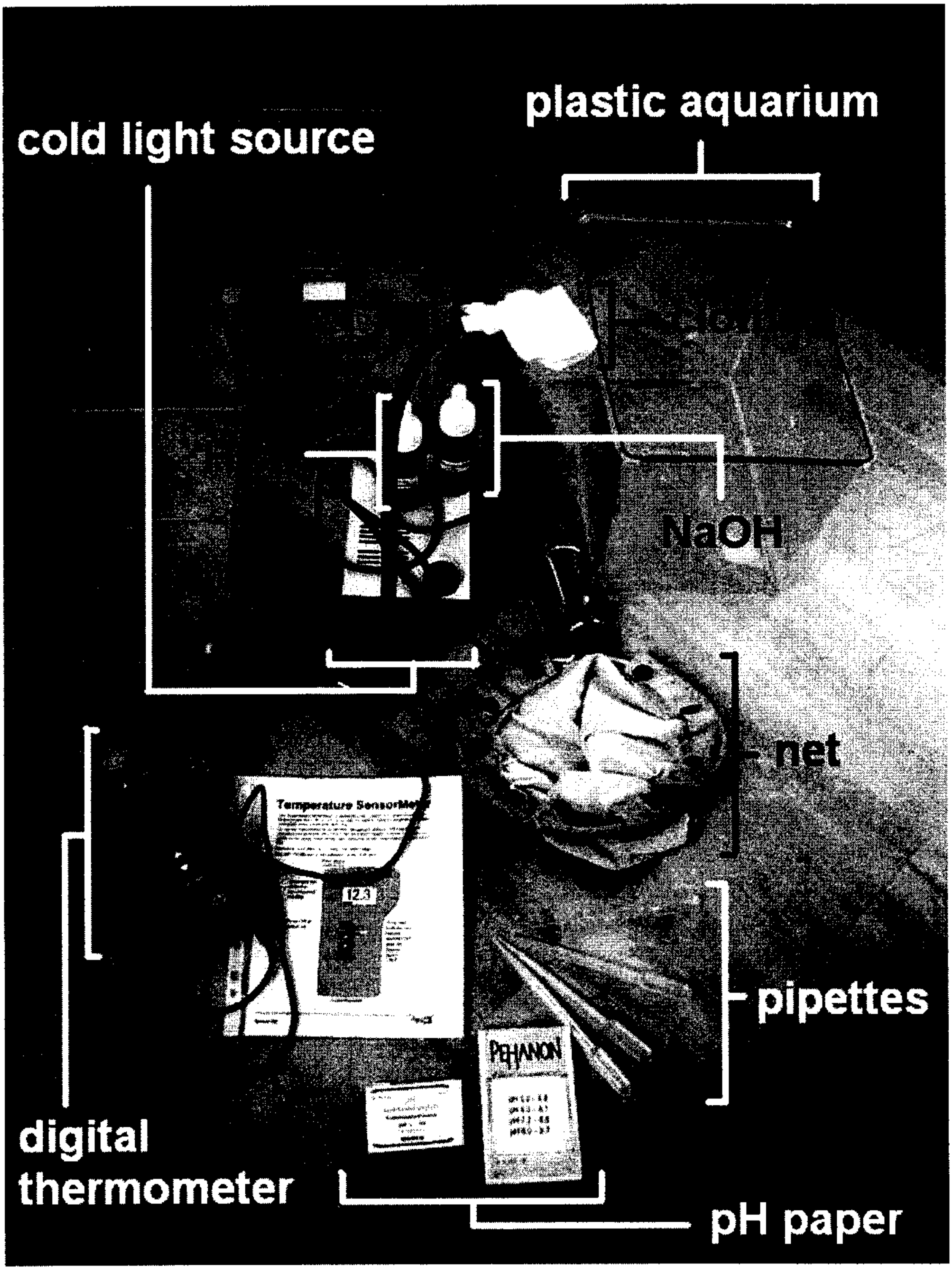
- Cold light source
- Small white cloth
- Rubber band

Additional apparatus for temperature investigation:

- 2 litre glass measuring cup
- Microwave oven
- Full tray of ice cubes

Additional apparatus for pH investigation:

- 150 ml bottle of 1M hydrochloric acid
- 150 ml bottle of 1M sodium hydroxide
- Litmus paper (to measure pH)


plastic aquarium
cold light source
NaOH
net
pipettes
digital
thermometer
pH paper
Temperature SensorMeter
12.3
PEHANON

6. Method

1. 1.225 litres of pond water were poured into the testing aquarium so that the water was 2 cm deep.
2. The water temperature was measured with the digital thermometer and was constant at 21.5°C (except in the temperature investigation, which used different water temperatures).
3. One dragonfly nymph was extracted from its home aquarium and placed carefully inside the testing aquarium.
4. The starting ring was placed around the nymph to trap it in the centre of the aquarium to set a constant starting point for movement.
5. (See variable-specific trial.)
6. The starting ring was removed, and immediately timing was started with the stopwatch program on the computer.
7. Within a two-minute period, every time the nymph started moving and stopped moving, the stopwatch was clicked. The number of turns made by the larva in movement was also recorded. (See variable-specific trial for more details.)
8. The nymph was then removed from the testing aquarium and placed inside the Tupperware container (containing tap water) to ensure that the same animal would not accidentally be tested again as a different sample.
9. This process (steps 3-8) was repeated with another 9 larvae from the 2 home aquaria.
10. The nymphs were all returned to their home aquaria.

(A) Light investigation

I) Full light trial

5. The cold light source was activated and shined in the nymph's direction.
7. As the larva moved, the light was aimed to constantly follow it.

II) Medium light trial

5. The end of the cold light source was covered with a white cloth, held in place by a rubber band, activated, and shined in the nymph's direction.
7. As the larva moved, the light was aimed to constantly follow it.

III) Dim light trial

5. A ceiling lamp at the back of the testing room was activated.
7. (No further details)

(B) Temperature investigation

I) Cold water trial (10.2°C)

2. Two ice cubes were dropped into the water and measured the water's temperature with the digital thermometer. This was repeated until the water temperature remained constant at 10.2°C.
5. A ceiling lamp at the back of the testing room was activated.
7. (No further details)

II) Warm water trial (31.3°C)

1. Approximately 2 litres of pond water was heated to 40°C in the microwave oven.
2. The temperature of the water was measured with the digital thermometer periodically until the temperature reached 31.3°C (approx. 30°C).
5. A ceiling lamp at the back of the testing room was activated.
7. (No further details)

(C) pH investigation

I) Acid trial (pH 4.9)

1. Six drops of hydrochloric acid were dropped into the testing aquarium, the water was mixed, and the pH was measured using litmus paper. This was repeated until the pH reached 4.9.
5. A ceiling lamp at the back of the testing room was activated.
7. (No further details)

II) Alkaline trial (pH 8.9)

1. Six drops of hydrochloric acid were dropped into the testing aquarium, the water was mixed, and the pH was measured using litmus paper. This was repeated until the pH reached 4.9.
5. A ceiling lamp at the back of the testing room was activated.
7. (No further details)

7. Data

7.1. Collection:

See Appendix

7.2. Processing

1) Bar charts were made to show the data in relation to each other.

2) Z-tests were used to determine the significance of the differences between the means of the sets of data for the investigated variables.

To calculate each critical value, means, the counts of raw values and standard deviations were needed.

3) Since significant differences would be sought within 5 %, the critical value was set as ±1.96.

Count of raw values (for all data sets):

$n = 10$

Mean:

$$\bar{x} = \frac{\sum \text{raw values}}{n}$$

Standard deviation:

$$\sigma = \sqrt{\frac{\text{sum of squared deviations}}{n - 1}}$$

All standard deviations were calculated using a TI-83 Plus graphics calculator. They are listed in the data tables.

Z-test:

$$z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

z = critical value

x = mean

σ = standard deviation

n = count of raw values

Level of significance:

For the z-test degrees of freedom are not required as they are for t-tests since the z-score of 1.96 is used for 5 %. (Gaten) For my experiment this means that a critical value greater than 1.96 or lower than -1.96 represents a significant difference.

Sample z-test calculation:

Between data sets of the total duration of movement of dragonfly nymphs under medium light and full light:

$$z = \frac{56.14 - 53.98}{\sqrt{\frac{16.83^2}{10} + \frac{20.20^2}{10}}}$$

$z = \underline{\underline{0.260}}$

Data tables for light investigation showing total moving time, number of directional changes and number of episodes in dragonfly nymphs

Dim Light

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	36,4	4	10
2	45,8	5	10
3	18,5	2	6
4	34,9	7	13
5	65,5	3	12
6	27,3	2	6
7	55,5	7	15
8	34,5	3	9
9	82,6	6	11
10	58,6	4	8

Mean	45,96	4,3	10
Standard deviation	18,50	1,79	2,76

Medium Light

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	53,10	3	8
2	63,80	4	6
3	60,90	4	10
4	82,50	2	5
5	55,70	4	10
6	23,90	2	11
7	74,40	3	9
8	32,30	2	6
9	50,20	4	17
10	64,60	1	7

Mean	56,14	2,9	8,9
Standard deviation	16,83	1,04	3,30

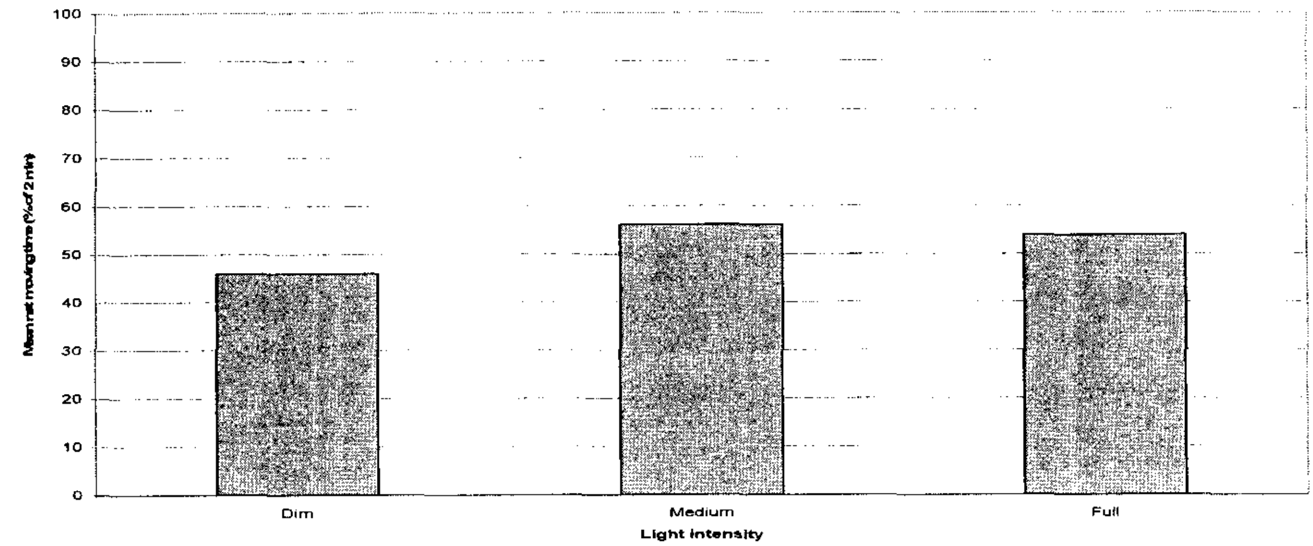
Full Light

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	23,95	9	5
2	37,06	7	12
3	45,34	7	12
4	31,96	10	6
5	64,87	10	11
6	51,75	14	13
7	92,38	5	5
8	51,63	6	6
9	80,10	14	12
10	60,80	3	8

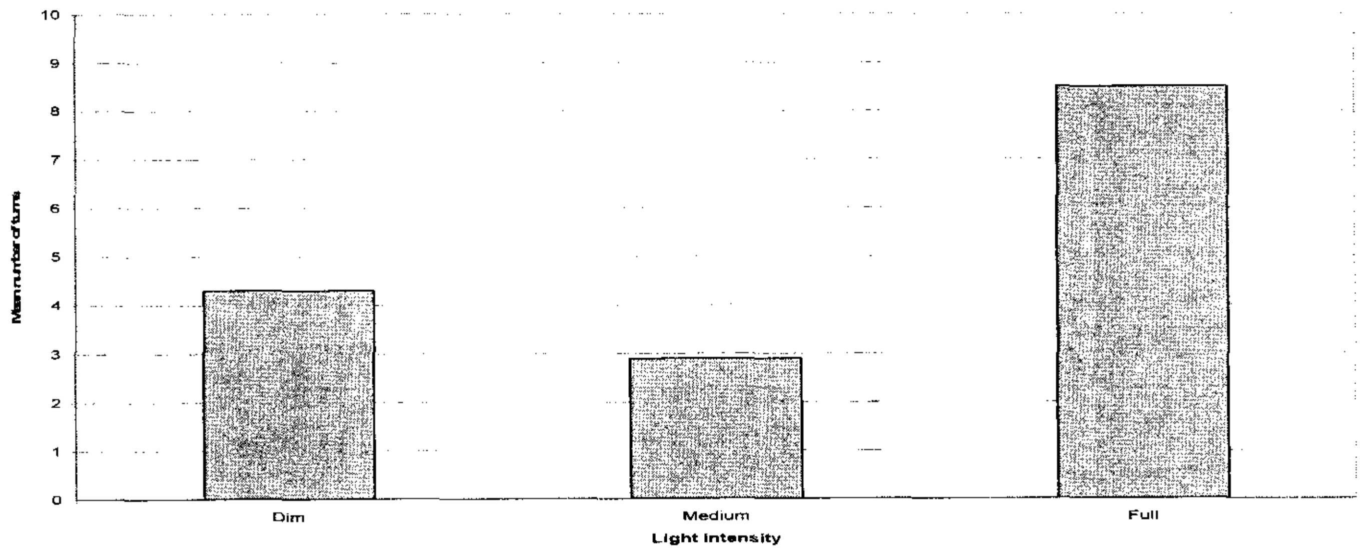
Mean	53,98	8,5	9
Standard deviation	20,20	3,44	3,13

Bar charts for light investigation

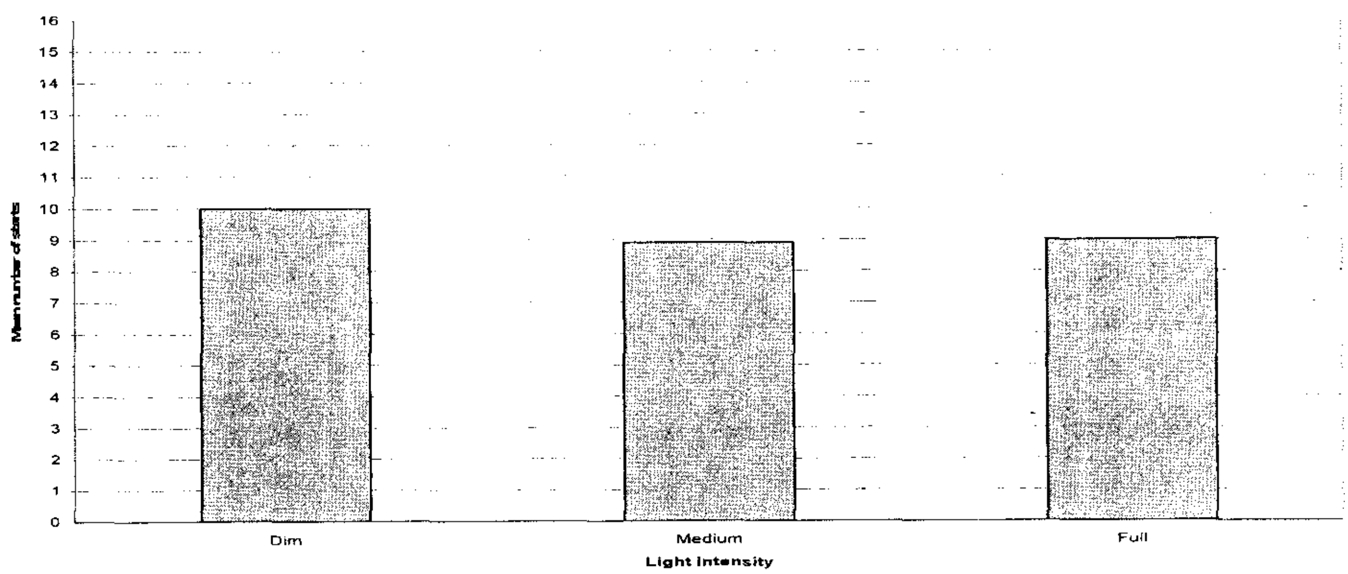
Mean net duration of movement of dragonfly nymphs at different light intensities



Mean number of directional changes made by dragonfly nymphs at different light intensities



Mean number of episodes of movement made by dragonfly nymphs at different light intensities



Data tables for temperature investigation showing total moving time, number of directional changes and number of episodes in dragonfly nymphs

10.2°C

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	47,90	6	7
2	21,20	2	7
3	69,30	8	7
4	54,70	5	5
5	51,80	1	7
6	71,60	3	9
7	47,70	7	8
8	49,20	1	5
9	88,70	4	3
10	59,30	7	7

Mean	56,14	4,4	6,5
Standard deviation	17,06	2,46	1,63

21.5°C

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	36,40	4	10
2	45,80	5	10
3	18,50	2	6
4	34,90	7	13
5	65,50	3	12
6	27,30	2	6
7	55,50	7	15
8	34,50	3	9
9	82,60	6	11
10	58,60	4	8

Mean	45,96	4,3	10
Standard deviation	18,50	1,79	2,76

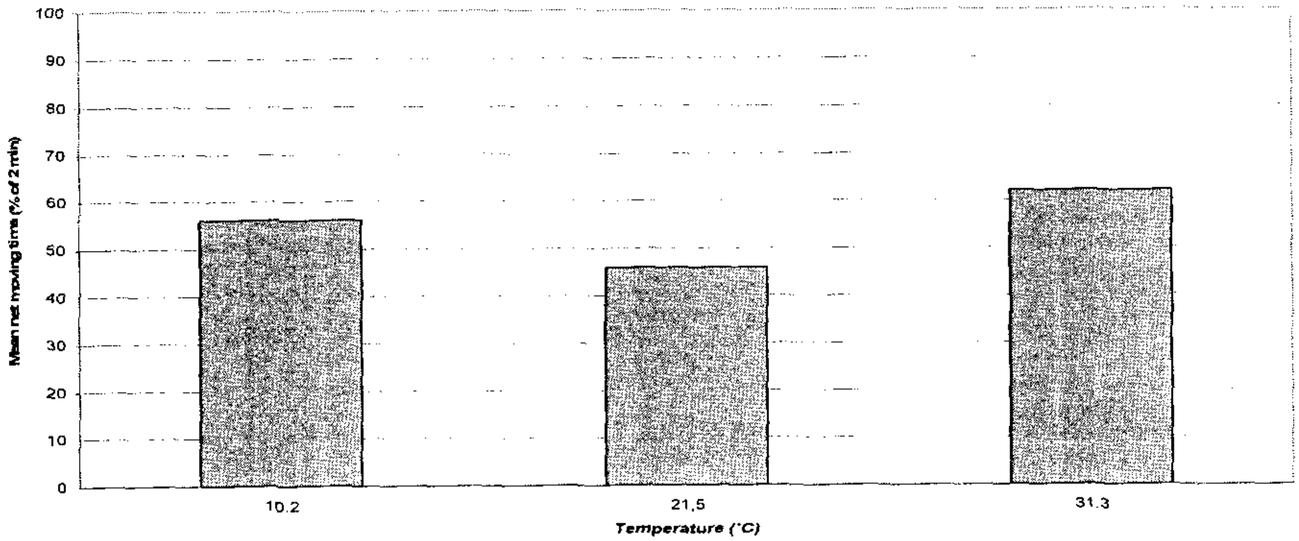
31.3°C

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	70,30	3	18
2	49,20	7	10
3	33,20	6	21
4	82,10	7	13
5	73,60	3	14
6	58,80	1	21
7	50,30	4	16
8	77,50	6	15
9	80,40	4	13
10	45,50	2	14

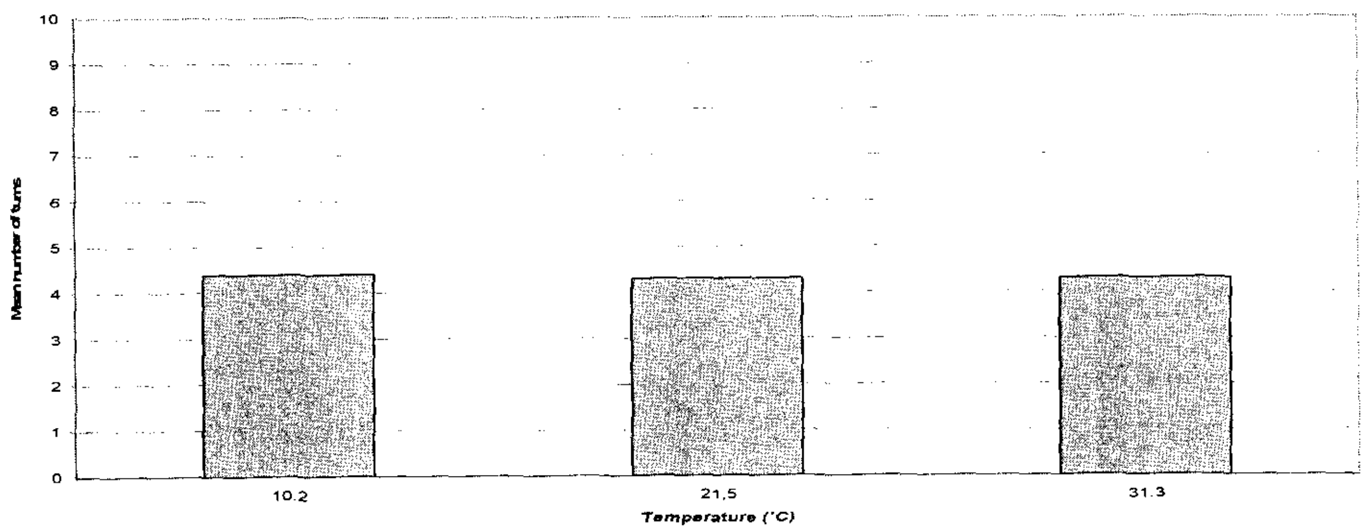
Mean	62,09	4,3	15,5
Standard deviation	16,12	2,00	3,38

Bar charts for temperature investigation

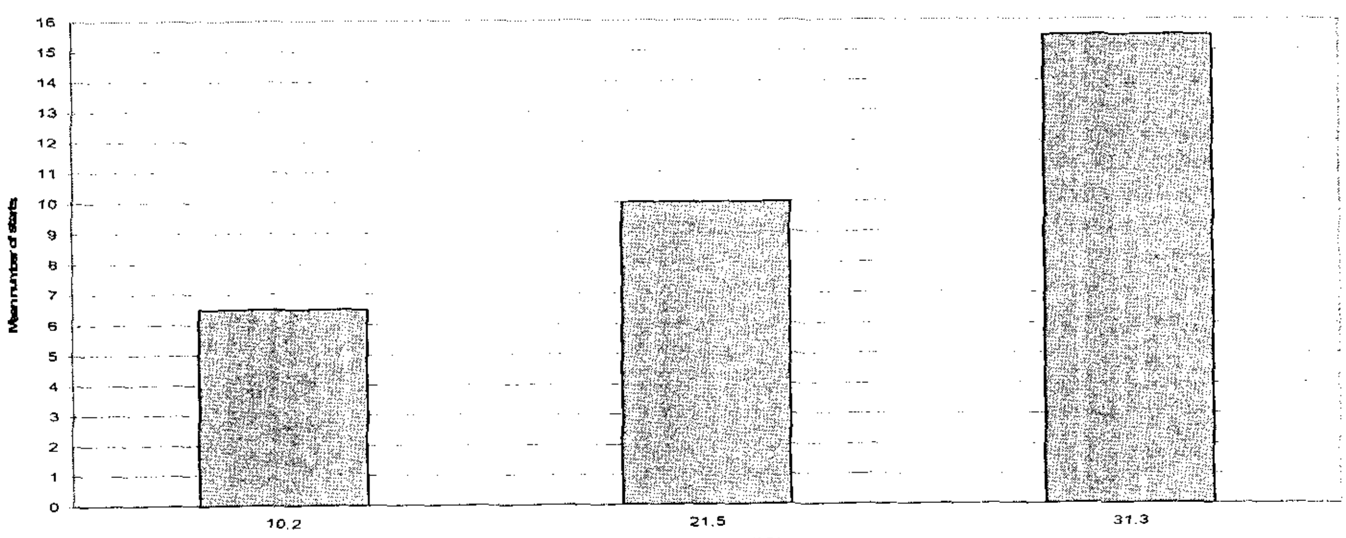
Mean net duration of movement of dragonfly nymphs at different temperatures



Mean number of directional changes made by dragonfly nymphs at different temperatures



Mean number of episodes of movement made by dragonfly nymphs at different temperatures



Data tables for pH investigation showing total moving time, number of directional changes and number of episodes in dragonfly nymphs

pH 4.9

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	27,20	4	8
2	12,60	2	7
3	53,70	7	15
4	36,90	6	15
5	26,40	2	12
6	23,40	4	10
7	43,10	5	20
8	44,50	4	20
9	80,00	3	8
10	61,00	3	16
Mean	40,88	4	13,1
Standard deviation	19,07	1,55	4,59

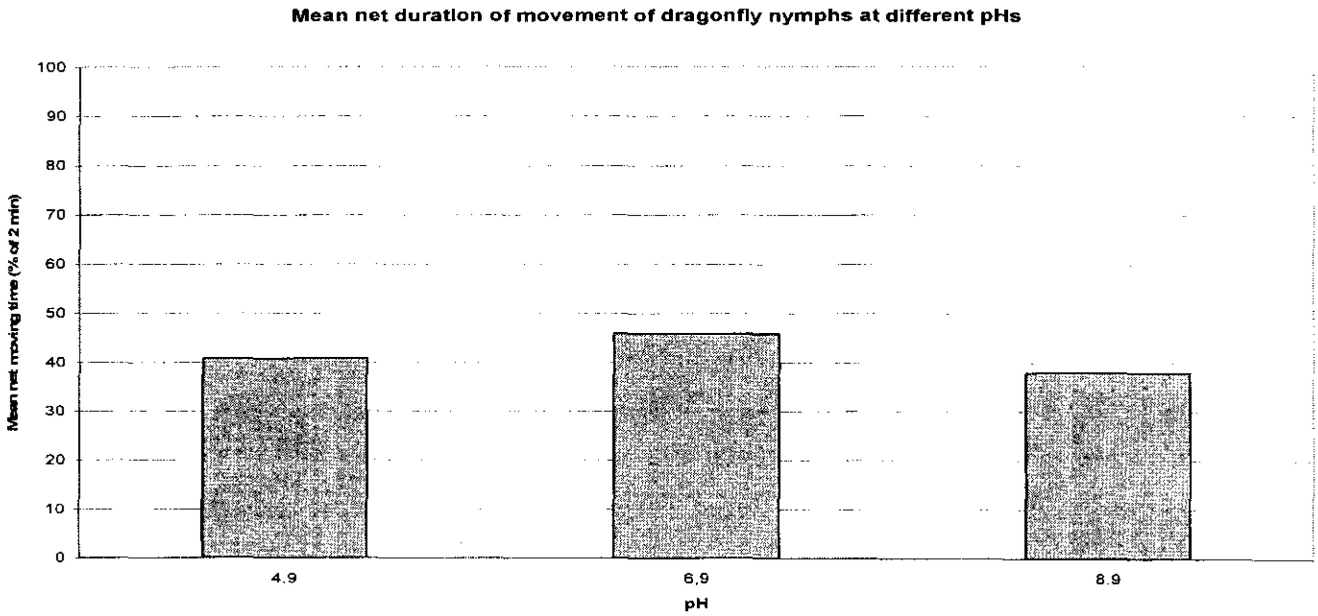
pH 6.9

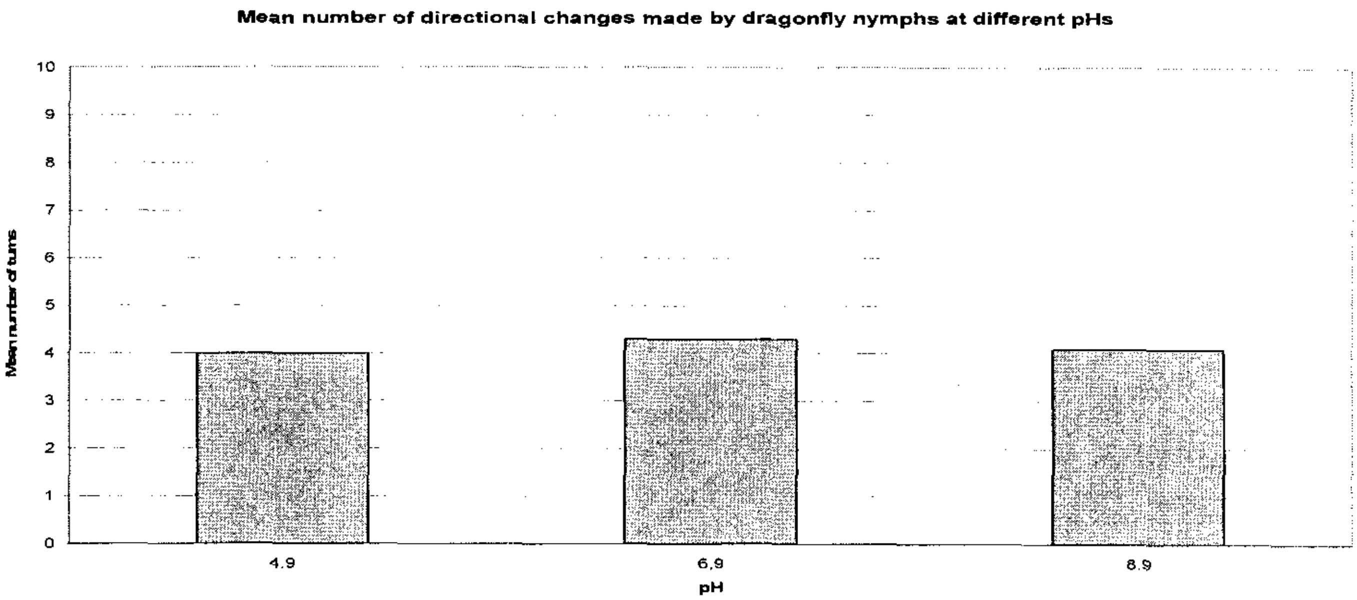
Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	36,40	4	10
2	45,80	5	10
3	18,50	2	6
4	34,90	7	13
5	65,50	3	12
6	27,30	2	6
7	55,50	7	15
8	34,50	3	9
9	82,60	6	11
10	58,60	4	8
Mean	45,96	4,3	10
Standard deviation	18,50	1,79	2,76

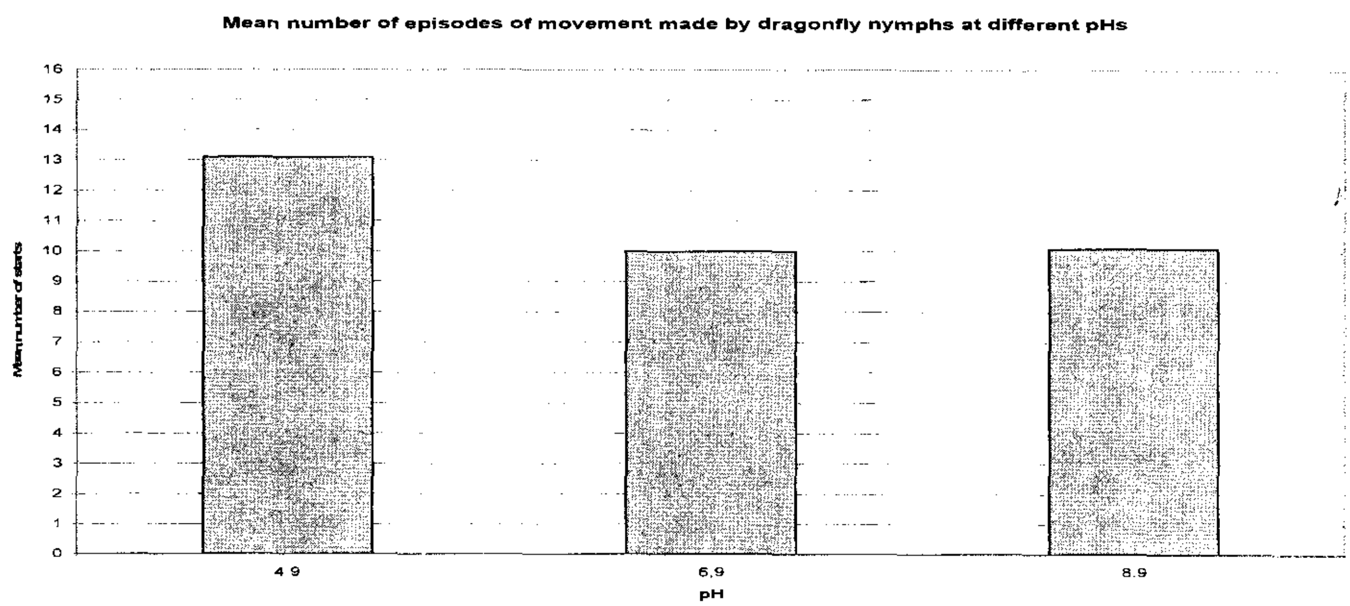
pH 8.9

Sample	Total moving time (percentage of 2 min)	Number of turns	Number of starts
1	33,70	5	16
2	10,70	1	5
3	11,70	3	6
4	41,30	5	10
5	42,10	5	9
6	25,50	4	12
7	44,00	4	13
8	44,60	4	10
9	79,40	4	10
10	47,00	6	10
Mean	38,00	4,1	10,1
Standard deviation	18,75	1,30	3,01

Bar charts for pH investigation







Z-test values for the light investigation:

Mean duration of movement (% of 2 min)

Full light - medium light	Medium light - dim light	Full light - dim light
0.260	1.287	0.926

Mean number of directional changes

Full light - medium light	Medium light - dim light	Full light - dim light
4.928	2.139	3.425

Mean number of episodes of movement

Full light - medium light	Medium light - dim light	Full light - dim light
0.070	0.809	0.758

Z-test values for the temperature investigation:

Mean duration of movement (% of 2 min)

31.3°C - 21.5°C	21.5°C - 10.2°C	31.3°C - 10.2°C
2.079	1.279	0.802

Mean number of directional changes

31.3°C - 21.5°C	21.5°C - 10.2°C	31.3°C - 10.2°C
0.000	0.104	0.100

Mean number of episodes of movement

31.3°C - 21.5°C	21.5°C - 10.2°C	31.3°C - 10.2°C
3.986	3.453	7.584

Z-test values for the pH investigation:

Mean duration of movement (% of 2 min)

8.9 - 6.9	6.9 - 4.9	8.9 - 4.9
0.956	0.605	0.341

Mean number of directional changes

8.9 - 6.9	6.9 - 4.9	8.9 - 4.9
0.286	0.401	0.156

Mean number of episodes of movement

8.9 - 6.9	6.9 - 4.9	8.9 - 4.9
0.077	1.830	1.728

7.3. Discussion

1. Duration of movement:

a) In the light investigation, the data obtained shows that the mean duration of movement of dragonfly nymphs was shortest in dim light conditions (45.96 %) and longest in medium light conditions (56.14 %), followed closely by full light conditions (53.98 %). The z-tests did not show any significant differences between the result sets.

b) In the temperature investigation, the mean duration of movement was longest at 31.3°C (62.09 %), shortest at 21.5°C (45.96 %) with 56.14 % at 10.2°C. The z-tests showed a significant difference between the result sets for 31.3°C and 21.5°C.

c) In the pH investigation, the nymphs moved the longest at pH 6.9 (45.96 %) and the least at pH 8.9 (38.00 %), moving 40.88 % of the time at pH 4.9. There were no significant differences between any of the result sets.

2. Directional changes:

a) At full light intensity, there were significantly more directional changes (8.5) than in medium (2.9) and dim light (4.3) conditions. The difference between the mean number of turns under medium and dim light was also significant.

b) In the temperature trials, the nymphs made a mean of 4.4 turns at 10.2°C, 4.3 turns at 31.3°C, and 4.3 turns at 21.5°C. No differences were significant.

c) In the pH investigation, the number of turns was similar at all pHs (4 at pH 4.9, 4.3 at pH 6.9 and 4.1 at pH 8.9). There were no significant differences between the data sets.

3. Episodes of movement:

a) In the light investigation, dim light conditions saw the highest mean number of starts (10), followed by full light (9) and medium light (8.9). There were no significant differences.

b) In the temperature investigation, there were the most starts at 31.3°C (15.5) and the fewest at 10.2°C (6.5), with 10 starts at 21.5°C. There were significant differences between all of the data sets.

c) In the pH trials, the highest mean number of starts was at pH 4.9 (13.1), followed by values of 10 and 10.1 at pH 6.9 and pH 8.9 respectively. There were no significant differences between the data sets.

7.4. Interpretation

In the light investigation, the number of directional changes varied greatly within the 3 different light intensities. It was interesting to see that at full light, the nymphs turned extremely often, perhaps as an instinctive attempt to evade predators (similar to the zigzag flight path of jackrabbits [Ballard et al.])
Under medium light conditions, the nymphs made the fewest turns, which may be due to a relative lack of immediate stress that would have been caused by exposure to potential predation.
The numbers of movement episodes under full and medium light were slightly lower than that in the dim light trial. This leads me to the careful assumption that the data supports the hypothesis that dragonfly nymphs would react to stressful conditions (i.e. intense light) with fewer stops. It must be

recognized, however, that the differences between the mean numbers of movement episodes in the light investigation were not statistically significant.

The significant difference between the duration of movement in the dragonfly nymphs at 31.3°C and 21.5°C in the temperature investigation could indicate that dragonfly nymphs are sensitive to temperature change within the used range. It could also indicate, however, that, referring to the studies of Pandian et al. (1979), warm water temperature promotes hunting behaviour. This is also supported by the significantly large number of movement episodes (starts and stops) observed at 31.3°C; in that trial, the nymphs may have stopped often to search (by visual, olfactory, auditory or tactile means) for prey. This analysis supports the hypothesis, which claims that "dragonfly larvae are likely to move…with…fewer stops…under stressful conditions", while here, the opposite is the case.

The lack of significant differences between the results of movement duration, number of directional changes and number of movement episodes in the pH investigation indicates that pH does not affect any of the given dependent variables.

8. Evaluation

The investigations were carried out in ways that produced unreliable data. One major reason for this is that only 10 specimens were tested in each trial. For a statistically representative study, many more samples (e.g. 100) would have been necessary for each trial.

The dragonfly nymphs may have not been sensitive enough to environmental change for the differences in the independent variables to have significant effect on their movement. A useful improvement to these experiments could be to include more extreme (high and low) levels of light intensity, temperature and pH in the respective trials.

In the light investigation I divided the three levels of light intensity between "Dim Light", "Medium Light" and "Full Light". To be able to compare my study with research conducted by others, it would have been necessary to use a standard system of measuring light intensity with units, i.e. using a light meter.

I initially considered using a common incandescent lamp with multiple power settings, but forewent it because it would have produced heat, interfering with the controlled variable of temperature. So I used a cold light source with unfortunately only one setting. Ideally I would have used a cold light source with multiple power settings in order to ensure an equal difference between all light intensities used.

Using the same dragonfly nymphs for multiple experiments could have adversely affected the validity of the results due to the specimens tiring or their physical condition otherwise deteriorating over time, due to less-than-optimal keeping conditions of the specimens and minimal knowledge of their needs on my part.

Identifying individual specimens (e.g. by marking them with a coloured substance) would have made the data more consistent and enabled matched pair analyses.
In addition, it was not possible to identify the family or species of the specimens, which were of the suborder *Anisoptera* (Encyclopædia Britannica, 2007). It would have been useful to ensure that all of the specimens were of one species for reasons of standardization, and to be able to compare my study with related ones.

The lab results may be different from those that would have been obtained in a field study for many reasons. One cause of discrepancy could be that the sides and bottom of the aquaria used were of a different texture than is typical in a lake or pond (i.e. smooth rather than muddy or rough). Similarly, the space available in dragonfly nymphs' natural habitat is much greater, wider and deeper than the aquaria they were kept and tested in. The direct light used in the experiments was not diffused by plants or particles in the water as it likely would have in the nymphs' natural environment. Other researchers have noted that natural responses cannot always be accurately predicted in controlled experiments. In "Test Chambers and Test Procedures for In Situ Toxicity Testing with Zooplankton" (1999), Anabela Maria Maia Pereira et al. urged caution in extrapolating field effects from laboratory results.

9. Conclusion

According to the data obtained over the course of the investigations and the significance of the differences between the data, the duration of movement by dragonfly nymphs increases as temperature increases, but remains unaffected by variations in the other independent variables, light intensity and pH. The data therefore only partially supports the hypothesis that dragonfly nymphs move for longer periods under extremes of light intensity, temperature and pH. The positive correlation between temperature and duration of movement suggests that warm water temperatures promote hunting behaviour, in which dragonfly nymphs are more physically active in searching for prey.

The number of directional changes made by the dragonfly nymphs increases with higher light intensity, a phenomenon likely due to a flight reaction triggered by visible exposure. This supports the hypothesis that dragonfly nymphs turn more often in movement under stressful conditions. The number of directional changes is not, however, affected by changes in temperature or pH.

The number of episodes of movement is unaffected by varying light intensity and pH, but increases with higher temperature. This does not totally support the hypothesis that dragonfly nymphs start and stop movement less often under stressful conditions.

Overall there is no pattern within that data consistent enough to draw a solid conclusion. Analysis was limited in part by questions left unresolved by research, such as the common conditions of dragonfly nymphs' natural habitat. Knowledge of this would have been required to estimate which conditions could be considered stressful for the nymphs.

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11. Appendix: Raw Data

Light Investigation

Dragonfly nymph movement under dim light (base conditions)

Sample 1

Click no.	Time of stopwatch click (min : sec , 1/100 sec)	Percentage of total time passed	Interval between clicks (min : sec , 1/100 sec)	Interval percentage
1	00:00,9	0,80	00:00,9	0,80
2	00:02,7	2,20	00:01,8	1,50
3	00:05,1	4,20	00:02,4	2,00
4	00:08,2	6,80	00:03,1	2,60
5	00:09,1	7,60	00:00,9	0,70
6	00:10,3	8,60	00:01,2	1,00
7	00:13,4	11,10	00:03,1	2,60
8	00:16,5	13,70	00:03,1	2,60
9	00:43,8	36,40	00:27,3	22,70
10	00:51,5	42,80	00:07,7	6,40
11	00:52,2	43,40	00:00,8	0,60
12	01:00,1	50,00	00:07,9	6,60
13	01:14,4	61,80	00:14,2	11,80
14	01:23,0	69,00	00:08,6	7,20
15	01:24,6	70,40	00:01,6	1,30
16	01:30,3	75,10	00:05,7	4,80
17	01:31,9	76,40	00:01,6	1,30
18	01:36,4	80,20	00:04,5	3,70
19	02:00,2	100,00	00:23,8	19,80

Total moving time (percentage of 2 min)	36,40

# of turns = 4

Temperature = 21.5°C
pH = 6,9

# of starts = 10,0