**Supplementary files**

**Additional file 1**

**A screenshot of a graph

Description automatically generatedFigure S1** Mortality at 20°C in a) *Ae. albopictus* vs *Cx. pipiens* s.s./*Cx. torrentium* and b) *Ae. japonicus* vs *Cx. p. molestus*, and at 26°C in c) *Ae. albopictus* vs *Cx. pipiens* s.s./*Cx. torrentium* and d) *Ae. japonicus* vs *Cx. p. molestus* under intraspecific competition to test which larval density is adequate for interspecific competition ratios. In blue is the percentage of mortality for a density of 30 larvae.

d))

c))

a))

b)

**Statistics:** Data was not normally distributed therefore a Friedman test was conducted, yielding non-significant results.

**Additional file 2**

A screenshot of a video game

Description automatically generated

d))

c))

b)

a))

**Figure S2** Mortality at 20°C in a) *Ae. albopictus* vs *Cx. pipiens* s.s./*Cx. torrentium* and b) *Ae. japonicus* vs *Cx. p. molestus*, and at 26°C in c) *Ae. albopictus* vs *Cx. pipiens* s.s./*Cx. torrentium* and d) *Ae. japonicus* vs *Cx. p. molestus* under interspecific competition.

**Statistics**: Data was not normally distributed, therefore a Friedman test was conducted (P = 0.01). Multiple comparisons were not significant. Mortality was highest in interspecific combinations for the *Ae. albopictus* x *Cx. pipiens* s.s./*Cx. torrentium* combination while for *Ae. japonicus* x *Cx. p. molestus* mortality was higher interspecific at 20°C and intraspecific at 26°C.

**Additional file 3**

A screenshot of a video game

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**Figure S3** **Development time during interspecific competition between the different species**. a) competition between *Ae. albopictus* and *Cx. pipiens* s.s./*Cx. torrentium* at 20°C, b) *Ae. japonicus* and *Cx. p. molestus* at 20°C, c) *Ae. albopictus* and *Cx. pipiens* s.s./*Cx. torrentium* at 26°C, d) *Ae. japonicus* and *Cx. p. molestus* at 26°C.

d))

c))

a))

b)

**Statistics**: A two-way ANOVA was conducted to compare the development time for 50 % for the pupae to emerge. For the combination *Ae. albopictus* vs *Cx. pipiens* s.s./*Cx. torrentium* at 20°C, *Species* (F = 8.816 ; P = 0.007), *Larval ratio* (F = 4.691 ; P = 0.01), and its *Interaction* (F = 3.294 ; P = 0.04) are significantly different. Tukey’s multiple comparisons test showed a difference between interspecific 20 *Cx. pipiens* vs. 20 *Ae. albopictus* (P = 0.01), 20 *Ae. albopictus* vs. 15 *Cx. pipiens* (P = 0.005), 20 *Ae. albopictus* vs. 15 *Ae. albopictus* (P = 0.008), 15 *Cx. pipiens* vs. 10 *Ae. albopictus* (P = 0.05) (Supplementary file 3 Figure S3a). At 26°C, the *Larval ratio* (F = 4.545 ; P = 0.01), *Species* (F = 77.40 ; P < 0.001), and its *Interaction* (F = 3.298 ; P = 0.03) are significantly different. Tukey’s multiple comparisons test showed significant differences for 30 *Cx. pipiens* vs. 20 *Cx. pipiens* (P = 0.04), 20 *Cx. pipiens* vs. 10 *Cx. pipiens* (P = 0.005), and 20 *Cx. pipiens* vs. 20 *Ae. albopictus* (P < 0.001) (Supplementary file 3 Figure S3c).

The combination *Ae. japonicus* vs *Cx. p. molestus* at 20°C showed significant differences for factors *Species* (F = 139.3 ; P < 0.001) and *Interaction between species and larval ratio* (F = 6.569 ; P = 0.002). Tukey’s multiple comparisons test showed significant differences between 30 *Cx. pipiens* vs. 30 *Ae. japonicus* (P < 0.001), 20 *Cx. pipiens* vs. 20 *Ae. japonicus* (P < 0.001), 15 *Cx. pipiens* vs. 15 *Ae. japonicus* (P = 0.03), 10 *Cx. pipiens* vs. 10 *Ae. japonicus* (P < 0.001), 20 *Cx. pipiens* vs. 15 *Cx. pipiens* (P = 0.02) (Supplementary file 3 Figure S3b). At 26°C, the *Interaction between species and larval ratio* (F = 3.651 ; P = 0.003) and *Larval ratio* (F = 4.287 ; P = 0.01) were significantly different. Tukey’s multiple comparisons test showed a difference between the 30 intraspecific and 10 interspecific *Cx. pipiens* (P = 0.002) and 10 interspecific *Cx. pipiens* with 20 interspecific *Ae. japonicus* (P = 0.02) (Supplementary file 3 Figure S3d).

**Additional file 4**

*Ae. albopictus*x*Cx. pipiens* s.s./*Cx. torrentium*

A screenshot of a computer game

Description automatically generated

f)))

e)))

d)))

c)))

b)))

a))

*Ae. japonicus* x *Cx. pipiens bioform molestus*

**A screenshot of a computer screen

Description automatically generated**

h)))

g)))

i))

k))

j))

l))

**Figure S4** Pupal size of *Ae. albopictus*, *Ae. japonicus*, and *Cx. pipiens* at 20°C and 26°C under interspecific competition.

**Statistics**: A two-way ANOVA was conducted to compare the pupal size for *Ae. albopictus*, *Ae. japonicus* and *Cx.* *pipiens* at 20°C and 26°C under interspecific competition. For the area of the cephalothorax at 20°C, the factors *Species* (F = 15.70 ; p < 0.001) and *Species ratio* (F = 6.804 ; p = 0.001) are significantly different. Tukey’s multiple comparisons showed significant differences within *Culex pipiens* (from *Ae. japonicus* ratio) when comparing 30 vs 10 (p = 0.01), 20 vs 10 (p = 0.005) and 15 vs 10 (p = 0.005) *Cx. pipiens* ratios. Between the species, there was a significant difference between 20 *Ae. japonicus* vs *20 Cx. pipiens* (p = 0.05), and 15 *Ae. japonicus* vs 15 *Cx. pipiens* (Supplementary file 4 Figure S4a-b). At 26°C, only the factor species was significantly different (F = 7.525 ; p < 0.001) (Supplementary file 4 Figure S4c-d).

The abdominal length at 20°C was significantly different for factors *Species* (F = 22.02 ; p < 0.001) and *Species ratio* (F = 3.817 ; p = 0.02). Tukey’s multiple comparisons showed significant difference for 30 vs 10 *Cx. pipiens* (p = 0.01), and for all densities between *Ae. japonicus* vs *Cx. pipiens* (both ratios). At 26°C, only *Species* (F = 19.70 ; p < 0.001) was significantly different. The multiple comparisons showed a significant difference for 20 *Ae. japonicus* vs. 20 *Cx. pipiens* (p = 0.02), for 15 *Ae. japonicus* vs. 15 *Cx. pipiens* (p = 0.04), for 10 Ae. albopictus vs 10 *Cx. pipiens* (p = 0.003), and for *Ae. japonicus* vs. 10 *Cx. pipiens* (p = 0.01).

The abdominal width at 20°C shows a significant difference for factors *Species* (F = 27.67 ; p < 0.001) and *Species ratio* (F = 2.928 ; p = 0.05). The multiple comparisons is significantly different for *Cx. p. molestus* in 30 vs 10 (p = 0.03), 20 vs 10 (p = 0.02), and 15 vs 10 (p = 0.03) and all species ratios. At 26°C, *Species* was significantly different (F = 36.02 ; p < 0.001). Tukey’s multiple comparisons test showed significant differences for *Ae. albopictus* between 20 vs 10 larval densities (p = 0.03), 30 *Ae. albopictus* vs 30 *Cx. pipiens* (p = 0.03), 15 *Ae. albopictus* vs 15 *Cx. pipiens* (p < 0.001), and 10 *Ae. albopictus* vs 10 *Cx. pipiens* (p < 0.001).

**Additional file 5**

**Tabel S1: Coefficient of variation of the pupal growth of *Ae. albopictus*, *Ae. japonicus, Cx. pipiens* s.s./*Cx. torrentium*, and *Cx. p. molestus.***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **T [°C]** | **Cephalothorax area [mm²]** | **Abdominal length [mm]** | **abdominal width [mm]** |
| *Ae. albopictus* | 20 | 0.35 | 0.19 | 0.06 |
| *Ae. albopictus* | 26 | 0.28 | 0.18 | 0.07 |
| *Ae. japonicus* | 20 | 0.48 | 0.26 | 0.07 |
| *Ae. japonicus* | 26 | 0.33 | 0.22 | 0.05 |
| *Cx. pipiens* s.s./*Cx. torrentium* | 20 | 0.30 | 0.17 | 0.05 |
| *Cx. pipiens* s.s./*Cx. torrentium* | 26 | 0.24 | 0.16 | 0.06 |
| *Cx. p. molestus* | 20 | 0.44 | 0.26 | 0.06 |
| *Cx. p. molestus* | 26 | 0.22 | 0.18 | 0.05 |

**Additional file 6**

A screenshot of a computer screen

Description automatically generated  
**Figure S5** Total distance moved, velocity and body contact measured during two minutes for different larval ratios in the *Ae. albopictus* and *Cx. pipiens* s.s./*Cx. torrentium*, and *Ae. japonicus* and *Cx. p. molestus* combinations.

c))))

b))))

a)))

**Statistics:** Data of the distance moved was sine transformed to assume normal distribution and a two-way ANOVA showed no significant differences between the species combinations and ratios. Data on the velocity violated normality, therefore multiple Mann-Whitney tests were conducted, showing no significant differences. For body contact a two-way ANOVA was conducted, showing a significant difference for the factor *Species combination* (F = 40.17 ; p < 0.001). Tukey’s multiple comparisons test showed significant differences between both species combinations for the ratios 6A:12C (p = 0.001), 9A:9C (p = 0.006), 12A:6C (p = 0.01), and 18A:0C (p = 0.004).

**Additional file 7**

*Ae. albopictus*x*Cx. pipiens* s.s./*Cx. torrentium*A screenshot of a computer screen

Description automatically generated

f)

e))))

d))))

c))))

b))))

a))))

*Ae. japonicus* x *Cx. pipiens bioform molestus*

A group of lines on a black background

Description automatically generated

l)

k))))

j)

i))))

h))))

g))))

**Figure S6** Size corrected lipid, glycogen and protein content per pupae from interspecific competition ratios at 20°C and 26°C.

**Statistics:** For the lipids, a two-way ANOVA revealed significant difference for the factor *Species and temperature* (F = 33.21 ; p < 0.001) in the *Ae. albopictus* x *Cx. pipiens* s.s./*Cx. torrentium* combination. Tukey’s multiple comparisons test showed significant differences for 10 *Cx. pipiens* vs 10 *Ae. albopictus* (p = 0.02), 15 *Cx. pipiens* vs 15 *Ae. albopictus* (p = 0.02), and 20 *Cx. pipiens* vs 20 *Ae. albopictus* at 20°C(p = 0.006). *Ae. albopictus* has very high values at 20°C, with less lipids in intraspecific competition. At higher temperature, the lipid content drops, but an opposite trend is observed with more lipids in intraspecific competition. For *Cx. pipiens* the difference between 20 and 26°C was much smaller, but at higher temperatures the lipid content was also lower. The factor *Species and temperature* (F = 3.006 ; p = 0.05) was also significantly different in the *Ae. japonicus* x *Cx. p. molestus* combination, but no differences were found in the Tukey multiple comparisons. They showed higher lipid content at higher temperatures, with highest differences in *Cx. pipiens*. *Aedes japonicus* showed higher lipid contents intraspecifically compared to interspecific competition.

The glycogen content in the *Ae. albopictus* x *Cx. pipiens* s.s./*Cx. torrentium* combination showed a significant difference for the factor *Species and temperature* (F = 48.28 ; p < 0.001). Tukey’s multiple comparisons test showed significant differences for 10 *Cx. pipiens* vs 10 *Ae. albopictus* at 20 (p < 0.01) and 26°C (p = 0.007), 15 *Cx. pipiens* vs 15 *Ae. albopictus* at 20 (p < 0.01) and 26°C (p = 0.002), 20 *Cx. pipiens* vs 20 *Ae. albopictus* at 20 (p = 0.01) and 26°C (p = 0.005), 30 *Cx. pipiens* vs 30 *Ae. albopictus* at 20 (p = 0.007) and 26°C (p < 0.01), and between 10 vs 20 *Ae. albopictus* at 20°C (p = 0.03). The glycogen content was always higher in *Ae. albopictus* compared to *Cx. pipiens*, with likewise to lipids a higher glycogen content intraspecifically at 26°C and lower glycogen content intraspecifically at 20°C for *Ae. albopictus*. In *Cx. pipiens*, variation between the two temperatures and competition was limited. The *Ae. japonicus* x *Cx. p. molestus* combination was sine transformed to assume normality. A two-way ANOVA showed no significant differences in glycogen content. The variation in glycogen was minimal for *Cx. pipiens*. *Aedes* *japonicus* exhibited a lower glycogen content at 26°C compared to 20°C.

The protein content in the *Ae. albopictus* x *Cx. pipiens* s.s./*Cx. torrentium* combination showed a significant difference for the factor *Species and temperature* (F = 15.99 ; p < 0.001). Tukey’s multiple comparisons test showed no significant differences. *Aedes albopictus* always had higher protein content in interspecific competition compared to intraspecific competition. *Culex pipiens* had some variety in interspecific competition ratios. Both species exhibit lower protein contents at higher temperatures. Data on the *Ae. japonicus* x *Cx. p. molestus* combination was sine transformed to assume normality. A two-way ANOVA showed no significant differences in protein content. Variation of protein content in *Ae. japonicus* was limited. *Culex pipiens* showed a higher protein content interspecifically compared to intraspecific competition.